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THE DIAGNOSTIC VALUE OF THE CEREBROSPINAL FLUID SUGAR CONTENT*

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WITHIN the last few years numerous papers have appeared dealing with the sugar values of the spinal fluid in disease. Inasmuch as there has been some variation in the conclusions reached as well as in the methods used, this paper is offered as an analysis of 122 fluids of our own series, and 434 other reported cases, all using the Folin-Wu technic, essentially as for blood sugar.

Our counts have all been made with a Fuchs-Rosenthal chamber after dilution of the fluid with one-tenth volume of 15 per cent acetic acid, mixed in a white blood cell counting pipette. Strictly normal fluids never run counts higher than 4 cells per cu. mm. by this method.

Globulin estimations were all made by adding one to two drops of spinal fluid to a watch glass full of saturated aqueous phenol solution, the clouding being read immediately and graded from negative to four-plus, the latter figure representing the equivalent of pure blood serum so used.

Sugar determinations were made according to the Folin-Wu supplemental method,[1] with precipitation by the tungstic acid reagents except in negative Pandy cases, where 0.2 c.c. of spinal fluid, measured in an accurate pipette, was used without protein precipitation.

DIAGNOSIS

In the reported cases the diagnoses are believed highly reliable. All tuberculous meningitis fluids are from cases proved by necropsy or by re-

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covery of the tubercle bacilli from the spinal fluid by the Roby sedimentation method,[2] which we highly recommend for its ease, simplicity, and reliability of results.

Purulent meningitides were all proved bacteriologically. All brain tumor fluids were from necropsied or operatively proved cases. Neurosyphilitic cases all had strongly positive Wassermann reactions. The other diagnoses were based either upon necropsy or the concurrent opinion of more than one consultant.

RESULTS

1. *Normals.*—Ten fluids were examined that could be considered normal. All had three cells or less and negative Pandy and Wassermann reactions. These were obtained from patients upon whom lumbar puncture was performed as a routine upon discharge from salvarsan treatment. The range of this group was from 60 to 90 mg. per 100 c.c., the median point being 83 mg. This figure is higher than most reported normals, perhaps due to the clinic hours being such that puncture was done about three hours after morning or evening mealtime.

TABLE I

VARIED NEUROLOGIC CONDITIONS

	DIAGNOSIS	CELL COUNT	PANDY	SUGAR MG. %
1	Neurotrophic foot	2	Neg.	114
2	Acute poliomyelitis	185	1+	111
3	Chorea	4	±	101
4	Glioma cerebri, necrosis	365	3+	93
5	Multiple sclerosis	3	1+	91
6	Amyotrophic lateral sclerosis	4	Neg.	91
7	Epilepsy	2	Neg.	90
8	Multiple sclerosis	1	1+	90
9	Acute poliomyelitis	33	2+	88
10	Spastic paraplegia	3	1+	87
11	Acute poliomyelitis	91	1+	80
12	Hysteria	2	Neg.	80
13	Brain tumor	230	3+	76
14	Pernicious anemia	1	Neg.	70
15	Multiple sclerosis	2	Neg.	70

TABLE II

PURULENT MENINGITIDES

NO.	DATE	CELLS	PANDY	SUGAR MG. %	TYPE
1		Cloudy	3+	25	Pneumococcus
2		3,000	3+	None	Pneumococcus
3		1,200	4+	None	Pneumococcus
4		20,000	4+	None	Streptococcus
5		Purulent	4+	20	B. Influenza
6		3,600	3+	None	B. Influenza
7		95	4+	20	Pneumococcus ventriculitis
8		65	4+	20	Late meningococcus
9	10/9	2,000	2+	None	Meningococcus meningitis;
	10/11	7,000	3+	None	treated, with recovery
	10/31	80	2+	57	
	11/12	9	±	64	
10	8/12	4,000	3+	None	Meningococci found
	8/19	300	3+	30	Meningococcus meningitis;
	8/23	220	3+	30	treated, with recovery
	8/27	25	2+	72	

2. *Nonneurologic conditions.*—This group comprised fifteen cases of uremias, hyperthyroidism, trichiniasis, active syphilis and the like, with cell counts of four or less and Pandy not over 1+. The range in this series was from 50 to 100 mg. with the median point at 80,—essentially the same as the normal group.

3. *Varied neurologic conditions.*—This group was composed of fifteen cases of nervous system disease in which there were too few examples of any one disease to classify it separately. The individual values will be shown in Table I. The group range was from 70 to 114 mg.; the median point was 90.

4. *Neurosyphilis.*—This group of fifteen cases of tabes, paresis, and cerebrospinal syphilis, all with strongly positive Wassermann reactions, showed cell counts ranging from 3 to 302 cells and Pandy from negative to 3+. As no relationship between either cell count or Pandy and sugar level could be found, detailed listing of the cases seems unnecessary. The group range was from 60 to 91; the median point 77 mg.

5. *Lethargic encephalitis.*—Of this interesting group of twenty cases with cell counts up to 191 and Pandy reactions up to 3+, only two cases fell above the range of our group of normals and varied nervous conditions. In these two cases the sugar values were 160 and 182 mg. respectively; but the latter was in a fluid obtained from a moribund patient who died within two hours and it probably reflects the preagonal rise in blood sugar that has been ob-

TABLE III

SPECIAL CASES

DATE	DIAGNOSIS	CELL COUNT	PANDY	SUGAR MG. %
	(1) Obstructive hydrocephalus. (severe)			
	Rt. lat. ventricle	60	4+	None
	Left lat. ventricle	45	4+	Trace
	Lumbar	445	Trace	60
	(2) Obstructive hydrocephalus.			
10/2	Ventricular	1	Neg.	133
10/2	Lumbar	2	Trace	100
10/4	Left ventricle	0	Neg.	125
10/4	Right ventricle	1	Neg.	114
	(3) Spinal sarcoma, lower dorsal level, pressure, myelitis.			
1/8	Lumbar	None	4+	60
1/4	Lumbar	14	4+	55
1/4	Cisterna magna	2	1+	55
	(4) Tetanus, intraspinal antitoxin, purulent aseptic reaction.			
8/18	(before antitoxin)	2	Neg.	77
8/19	(after antitoxin)	16,000 (pus)	4+	70
8/20		4,200	3+	63
8/22		40	1+	71
8/24		40	1+	120
	(5) Cerebral abcess, asceptic "sympathetic meningitis," sterile fluid.	1,600 (polys)	2+	70
	(6) Compound fracture of frontal bone, aseptic sympathetic meningitis, sterile fluid.	450 (polys)	1+	60

served in patients dying of other nondiabetic conditions. The group range was from 60 to 182; the median point, 81.

6. *Tuberculous meningitis.*—Twenty-one proved cases of this disease showed a range of from 10 to 50 mg. with the median point of the series at 30; figures approached by no other disease such as poliomyelitis, encephalitis, neurosyphilis or meningism in which the cerebrospinal fluids often show globulin and cytologic reactions like those in this disease.

7. *Purulent meningitis.*—Detailed report of this group is given in Table II. The range in all except recovered meningococcic cases was from 0 to 25, the majority showing only a faint unreadable trace.

In Table III are shown a group of cases of considerable interest in regard to the factors other than blood-sugar level, concerned with cerebrospinal fluid sugar. Cases 1, 2, and 3 all represent some obstruction to the flow of the cerebrospinal fluid. The absence of sugar in the ventricles in the first case, is probably due to residual infection. The fact that nearly normal sugar value was found in this case at the lumbar level and that the sugar values were essentially the same above and below the obstruction in Cases 2 and 3 indicates definitely that sugar enters the cerebrospinal fluid at all levels of the system.

Cases 4, 5, and 6 show fluids with varying degrees of purulent reaction without infection of the fluid itself and with normal sugar values, indicating

TABLE IV

	COOPE (quoted)	COOPE (own)	WILCOX LYTTLE	FOSTER	STOWE	TOTAL
			Normals			
Cases		3		22	10	35
Range		Av. 72		Av. 53	60-90	
Med. Pt.					83	
			Assorted Neurologic			
Cases	55	69	33		14	171
Range	25-110	43-111	40-99		70-114	25-114
Med. Pt.	73	72	59		90	71
			Neurosyphilis			
Cases		19			15	34
Range		44-102			60-91	44-102
Med. Pt.		70			77	73
			Nonneurologic			
Cases					14	14
Range					50-100	50-100
Med. Pt.					80	80
			Encephalitis Lethargica			
Cases	92	11	16	39	20	182
Range	30-110	54-94	51-121	50-113	60-182	30-182
Med. Pt.	79	75	80	74	81	77
			Tuberculous Meningitis			
Cases	27	12	19	11	21	32
Range	10-60	14-55	10-81	1-41	10-50	1-50
Med. Pt.	33	25	29	25	30	27
			Purulent Meningitis			
Cases				5	14	19
Range				0-	0-30	0-30
Med. Pt.				70	.01-	.01-

that the mere presence of pus cells up to 16,000 per cu. mm. does not diminish the sugar in the cerebrospinal fluid.

In Table IV, I have brought together my own series and those reported by Coope,[3] by Wilcox and Lyttle[4] and by Harold Foster[5, 6] together with summation of the entire groups, in tabular form.

CONCLUSIONS

1. While few compilations of figures seem available on normal fluids, the general trend of recent literature is to raise the figure ascribed to sugar content considerably above that given by the earlier French workers and by Foster, and place it in the neighborhood of 75 mg. per 100 c.c.

2. When series of cases of neurosyphilis, lethargic encephalitis and other neurologic and nonneurologic conditions, other than the meningitides, are examined, their deviation from the normal range and median point is found to be so slight as to be without diagnostic significance.

3. Tuberculous meningitis gives values so definitely above the purulent meningitis cases and so definitely below all other conditions that an almost certain diagnosis can be quickly made in this disease.

4. The occasional purulent fluids obtained after intraspinous therapy, in brain tumor cases with necrosis or hemorrhage near the ventricles, in sympathetic aseptic meningitis, etc., can be rapidly delimited by their normal sugar content from infective meningitis in which the causative organism may not be promptly found. The presence of pus cells alone in the cerebrospinal fluid does not reduce its sugar content.

5. Two of our cases are interesting in that obstruction to the flow of cerebrospinal fluid at the base of the brain in the one and at the lower dorsal spinal level in the other did not produce any essential difference in the sugar content above and below the lesion. This would suggest that the diffusion of sugar into the cerebrospinal fluid occurs throughout the cerebrospinal axis rather uniformly and not through the choroid plexus alone.

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DISCUSSION

Dr. Ward Burdick.—We have found the test for the sugar content of the cerebrospinal fluid a very useful procedure in the Children's Hospital, of Denver. Our findings correspond quite consistently with those of Dr. Stowe. In encephalitis the sugar is often, but not always, increased; in syphilis it is usually normal (70 to 80 mg. per 100 c.c. of cerebrospinal fluid), while in acute meningitides (tuberculous, pneumococcal, influenzal, streptococcal, etc.) it is invariably markedly reduced and in rare instances entirely absent.

Dr. John A. Kolmer.—Has Dr. Stowe studied the blood sugar in relation to variations in the spinal fluid sugar?

Has Dr. Stowe studied the fate of the sugar in the spinal fluid in tuberculous and meningococcus meningitis? Mestrezat and others have expressed the opinion that the sugar

is removed by the leucocytes but my own experiments have not substantiated this view and I was interested to observe that in some of his cases with sterile fluids but containing large numbers of leucocytes, the sugar content was within normal limits indicating that the cells do not receive the sugar but that its reduction in acute bacterial meningitis may be due to its utilization by the organisms.

Dr. A. S. Giordano.—I have noted with interest the normal variations found by Dr. Stowe for they closely correspond with mine and these figures differ from those published by others. I have had seven cases of tuberculous meningitis and of these, four had no sugar, one had 45 mg. and the others varied from 5 to 15 mg. per 100 c.c. All of these cases were proved to be tuberculous meningitis either at autopsy or by demonstrations of the tuberculous bacillus in the spinal fluid by smear or pig inoculation. My findings in a few cases of so-called encephalitis have been very unreliable, because the values fell within my normal limits. I believe that in order to establish the real value of this diagnostic procedure no case report should be accepted unless the diagnosis has been substantiated by demonstration of the causing organisms or by postmortem. I have more than once found that the clinical diagnosis of encephalitis was erroneous at autopsy even when such a diagnosis was made by a recognized neurologist. In general, I feel confident that given a clear spinal fluid with a sugar content below 45 mg. per 100 c.c. and absence of any demonstrable organisms, in all probability this is diagnostic of tuberculous meningitis. I do not believe that at present the determination of glucose in spinal fluid can be given any other diagnostic significance.

Dr. Stowe (closing).—"I was very much interested in the discussion, especially that of Dr. Giordano, whose results were similar to ours. We found no tuberculous meningitides with no sugar at all. In encephalitis lethargica I feel sure the range is not higher than that in normal fluids, and that the spinal fluid sugar has little diagnostic value in this condition. We have had few blood sugars to correlate with our spinal fluids. One patient with diabetic coma had 600 mg. of blood sugar and 500 mg. of spinal fluid sugar at the same time. As to the cause of the diminished sugar in the meningitides I have little to offer. I had thought that it might be due to the action of the cells present but this would seem disproved by the brain tumor and antitetanic serum reaction cases that had very high cell counts and normal sugar values. Against the idea of the bacteria using it up, some meningococcus cases with few organisms reduce the sugar to zero while a trace has been present in some pneumococcus cases with innumerable organisms. Also tuberculous meningitis contains relatively few organisms but the sugar is greatly reduced. Perhaps the answer is in altered permeability of the cerebrospinal vascular apparatus.

HOW CAN WE BEST PROMOTE THE OBJECTS CONTAINED IN ARTICLE TWO OF OUR CONSTITUTION?*

By Herman Spitz, M.D., Nashville, Tenn.

ARTICLE two of our constitution states the objects of our society. Without these we would have no bona fide reason for our existence. I will read these objects so that they will be fresh in our minds:

The objects of this society shall be:

"(1) To promote the practice of scientific medicine by a wider application of clinical laboratory methods to the diagnosis of disease.

*Read before the Fourth Annual Convention of the American Society of Clinical Pathologists, in Philadelphia, May 20-23, 1925.

"(2) To stimulate original research in all branches of clinical laboratory work.

"(3) To establish from time to time uniform standards for the performance of various laboratory examinations.

"(4) To elevate the scientific and professional status of those specializing in this branch of medicine.

"(5) To encourage a closer cooperation between the practitioner and the clinical pathologist."

Our active membership is limited to graduates of medicine from recognized medical schools, who have specialized in clinical pathology for at least three years and who devote the major portion of their time to clinical pathology. A question which naturally arises is this: As physicians, who specialize in the field of clinical pathology, are we equally interested in all of the objects as enumerated or are some of us interested in one of the objects more than in some of the other objects? Stated in another way—are you interested in promoting the closer cooperation between practitioner and clinical pathologist as much as or more than in the stimulation of original research; or is each of us interested in elevating the status of some questionable laboratory or individual as much as he is in establishing uniform standards in laboratory work? The answer depends upon the particular field of clinical pathology in which we are engaged.

THE CLINICAL PATHOLOGIST

The clinical pathologist is a peculiar combination of physician and laboratory worker. As a physician, he must be familiar with all of the medical sciences, he must be able to diagnose, to treat, and to prognose. He must be willing to share the responsibility of a given case with the physician who calls on him for help. As a pathologist, he must be familiar with the processes of disease, and with the aid of laboratory methods he must be able to distinguish between the normal and abnormal, both in structure and function, and be able to tell why such abnormality in function or structure or both occurs.

Stedman defines a clinician as a practicing physician as distinguished from a pathologist or laboratory worker. The same authority defines a pathologist as one versed in the special study of morbid changes associated with disease. I do not find a definition of the compound word clinical pathologist, other than the one given by the Advisory Advertising Committee of the American Medical Association, reported in the *Journal of the American Medical Association,* December 2, 1922. "A Clinical Pathologist is a manipulator of fixtures and inanimate substances." It would thus seem necessary that this society define the terms clinical pathology and clinical pathologist so that the world may learn that there is some difference between a clinical pathologist and a technician, whose most proficient accomplishment may be the performance of an albumin or sugar test on urine, or the developing of an x-ray plate.

A clinical pathologist must be a physician, primarily and basically. He must not only know medicine but he must also be familiar with the allied

sciences; namely, chemistry, physics, biology, botany, mathematics, et cetera, and upon this broad foundation he places his structure of pathologic knowledge. Not the mere adding of two liquids in a test tube but the ability to interpret in terms of body activity the result of such a simple procedure is the function of a clinical pathologist.

Let us then consider this definition:

Clinical pathology is the science of interpreting morbid processes as determined by means of various laboratory aids and correlating them with clinical symptoms.

In order to fulfill this requirement we see that a thorough clinical knowledge is of prime importance and he who attempts clinical pathology without clinical knowledge and experience is falling short of the possibilities within his field. May I refer to a few examples to more forcibly present this thought?

A dermatologist saw a small growth in the flexor surface of a finger. It was removed and sent to a pathologist for diagnosis. The pathologist reported a giant cell sarcoma and without seeing the patient advised amputation. Fortunately other opinions were sought and the neoplasm proved to be a giant cell tumor of the tendon sheath, a growth well known for its benign character. The lack of clinical experience on the part of the pathologist was almost productive of serious results.

Another case from a different pathologist showing lack of clinical experience proved more serious. An enlarged submaxillary node was removed and the pathologist reported a lymphosarcoma. X-ray treatment was instituted and serious burns resulted. When the wound refused to heal, consultation was sought and the removed gland proved to be inflammatory from infection around an unerupted tooth.

The dermatologist calls the clinical pathologist into consultation in many confusing cases. A particular case may present lesions suggestive of blastomycosis, lupus, epithelioma or gumma. The clinical pathologist suggests the proper tests and from this knowledge should direct the investigative work into the proper channels in order to make a diagnosis as early as possible and also to avoid unnecessary work, so that the patient may be saved needless expense.

The ophthalmologist calls us into consultation in obscure eye conditions. Has the patient an optic atrophy due to nephritis, lues, brain tumor or diabetes? The clinical pathologist again suggests the advisable tests.

The surgeons want the clinical pathologist present at the operation, so that he can see the tissue in situ as well as after it is removed. The clinical pathologist frequently decides for the surgeon how extensive his operative work should be. May I refer to another case? A man in the middle forties presented himself with an enlarged testicle, with a diagnosis by the surgeon of a carcinoma. It proved to be a gumma. Had the clinical pathologist been consulted prior to the operation, instead of after it, the patient would have been saved this mutilating operation.

Another case: a man thirty-eight years of age was advised by two surgeons to have his leg amputated, one surgeon's diagnosis being tuberculosis of the knee, the other, carcinoma. In this case the clinical path-

ologist was called in and he cleared the case by proving it to be luetic; the ulcerations of four years standing responding promptly to proper treatment.

The internist depends upon our judgment in numerous cases. A case was diagnosed as pernicious anemia by a very competent internist, the blood work being done by his office laboratory assistant. Not satisfied, this patient sought other advice and it remained for the clinical pathologist to show that the pronounced anemia in this case was due to constant oozing from hemorrhoids that had escaped the internist's attention.

But why go on with this enumeration? I am sure that each of you who is in active clinical pathologic work can recall similar cases. My object in briefly referring to these few cases is to emphasize in as forceful a manner as lies in my power, that clinical acumen must be the basis of the clinical pathologist's work. This is the main contention in my paper and all I have to say in this paper is to support this contention. With no intention to ignore, slight, or belittle the tremendous amount of invaluable work being done by clinical pathologists in research work, most of which is being carried on in special institutions, I hold that the clinical pathologists in the forward ranks, those in active practice of clinical pathology, who daily see the general practitioners and their patients are the ones who sell clinical pathology to the profession at large.

DISTRIBUTION OF CLINICAL PATHOLOGISTS

In an effort to classify clinical pathologists according to the nature of their work, I was amazed at their wide distribution. They are found in all fields of medical activity. An attempt was made to classify them according to hospital connections but this led into so many subclassifications that I abandoned this method. Then too, the Council on Medical Education and Hospitals of the American Medical Association has recently (during the present year) undertaken a very broad survey of the laboratory situation throughout the country. This survey was undertaken "with the knowledge and advice of the officers of the American Society of Clinical Pathologists" and several other national organizations. (Letter dated April 13, 1925, signed by secretary of council.) It is hoped that the result of this survey will do much to clarify not only the laboratory situation but also establish the clinical pathologists in charge of these laboratories as something superior to a mere manipulator of test tubes and other inanimate substances.

Clinical pathologists are found in hospitals, in private laboratories, and a large number, like myself, dividing their time between the hospital laboratory and the private laboratory.

In hospitals, we find the clinical pathologists as a rule engaged in routine work. Some devote some part of their time to research. There are a large number who devote all their time to research and to teaching, these usually being connected with special hospitals, institutions, and schools.

Other clinical pathologists are found in federal, state, county, or city public health work, either as director or in charge of health laboratories. Most of these find little or no time for private laboratory work.

Still other clinical pathologists are found in industrial organizations; such as food distributing organizations, large milk plants, insurance companies, et cetera.

INTEREST AND DUTIES OF CLINICAL PATHOLOGISTS

Let us examine the interest and duties of the clinical pathologist in these various connections and see if they harmonize with our objects. Basically, as stated before, each of these clinical pathologists must be a physician. However, we find that those clinical pathologists who come most frequently and constantly in contact with the general practitioner and specialists and their patients are the ones who are most vitally concerned with the medical application of laboratory diagnosis, while on the other hand those clinical pathologists who are in administrative positions, who seldom if ever come in personal touch with the physician and his patient are concerned primarily with the technical features of laboratory work.

If this general survey is tenable, and I so hold, we can readily see that each of these groups of clinical pathologists cannot be equally interested in each or all of our objects.

The first object is: To promote the practice of scientific medicine by a wider application of clinical laboratory methods to the diagnosis of disease.

We should expect those clinical pathologists connected with hospitals and in private laboratories to be most vitally interested in this object. Those, who like the health board laboratory men and those in other administrative positions seldom come in contact with patients, would have only a purely academic and passing interest in this object.

The second object is: To stimulate original research in all branches of clinical laboratory work.

In this field the clinical pathologists connected with the so-called teaching hospitals, research hospitals, and special hospitals and schools would find their special field of interest.

The third object is: To establish from time to time uniform standards for the performance of various laboratory examinations.

It remains to be seen whether "uniform standards" is the ideal in our laboratory work. A standard once accepted would usually mean that it is the best. Accepting it as such would remove all incentive to find a better method and so we would defeat object two, just considered. Those most interested would be the clinical pathologists connected with the practical, everyday, active practice of clinical pathology; those who come in constant contact with clinical material, such as, clinical pathologists connected with hospitals, special institutions, and private and group laboratories. May I suggest "optional standards" as a better term meeting the situation?

Object four is: To elevate the scientific and professional status of those specializing in this branch of medicine.

In my opinion, we have in this object the possibilities of doing some really constructive work. To elevate our standards is a worthy and laudable undertaking. However, the mere stating of this object will not achieve results. We must outline the various ways by which we hope to attain this elevation in status and then adopt definite rules of procedure to enforce them.

The code of ethics of the American Medical Association is our code. A number of items in that code are inapplicable to our society. Many rules of conduct are missing therefrom that could and should be incorporated in our code.

Dr. Sondern and his Committee on Standardization have laid before us a plan for standardization as affects the clinical pathologist alone. We must not overlook the fact that we have to deal with hospitals, with other practitioners, with other pathologists, with our employers and employees, with patients and with the public. We should have definite rules governing our various relationships, and to that end I propose that this society adopt a new code of ethics that will include all the valuable features contained in the code of the American Medical Association and such other rules of ethical procedure as may be found advisable. The code should cover rules on advertising, on contracts with hospitals, our relationship with our technical help, and all of our contacts.

Object five is: To encourage a closer cooperation between the practitioner and the clinical pathologist.

Those clinical pathologists in private laboratories, group laboratories, private and general hospitals, special hospitals and some special institutions are the ones most interested in this object because they are the ones who come more frequently in contact with the general practitioner and his patients. This comprises the largest group in the practice of clinical pathology.

MOTIVE

What is our motive in adopting these objects? Is it altogether altruistic or is there an element of selfishness in our motive? We may well ask ourselves and then answer because sooner or later we will be asked this very question. If we consider the first and last objects we see a rather suggestive parallelism. The first object is "To promote a wider application of clinical laboratory methods" and the fifth object is to promote a "closer cooperation between clinical pathologist and practitioner." In the minds of some, this may mean but one thing and that is a bid for more work from the practitioner.

That thought may be justified and should be justified. How? By a closer personal application to our work; by giving more thought to clinical application than to theoretical interpretation; by being a consultant and not a mere "manipulator of constants and inanimate substances." By being a clinician first and then a pathologist—by living up to our name in the order in which we are named, clinical pathologist.

NOTE: It is to be hoped that the results of this survey will be published by the council before all interest in it is lost.

DISCUSSION

Dr. Frank M. Huntoon.—Again I want to come to the defense of common sense. We are here for three reasons: first, to make a decent living, second, to be a good clinical pathologist, third, to educate the medical profession to appreciate good clinical pathology and good clinical patholgists. I am glad to see a paper brought forth that infers that there is a selfish motive. We all have that motive. It is through the duration of that motive that real good clinical pathologists are made. In the education of the physician we have to carry it a considerable way to reach the mutual benefit that is possible.

THE PHARMACOLOGY OF BENZYL ALCOHOL AND ITS ESTERS*

V. A Pharmacologic Investigation of the Effect of Sodium Benzyl Succinate "Benzycin" and Sodium Dibenzyl Phosphate "Benzyphos" upon the Respiratory and Cardiovascular Systems

By Charles M. Gruber, Ph.D., M.D., St. Louis, Mo.

In 1918 Macht[1] noted that benzyl benzoate and benzyl acetate produced a fall in arterial blood pressure in dogs and rabbits when injected subcutaneously, intramuscularly, intraperitoneally, or intravenously. Two years later he[2] reported favorable results with the use of benzyl benzoate clinically, in circulatory hypertension and angina pectoris. Recently Babcock[3] published a report on the use of benzyl benzoate in the treatment of over twenty cases of angina pectoris with, as he believes, beneficial results in six cases. Spach[4] likewise believes this drug to be beneficial in angina pectoris. In 1920 Mason and Pieck[5] published a report on a series of experiments performed by them, in which they found that benzyl benzoate injected intravenously in dogs was followed by a prompt, pronounced, and prolonged fall in blood pressure. This lowering of blood pressure, they believed, was due, not to its action upon the blood vessels, but to its action upon the heart.

Nielsen and Higgins,[6] in a series of papers reporting their results upon intravenous administration of benzyl compounds, support Macht's findings. In 1923 Macht[7] studied the pharmacologic and clinical effect of benzyl mandelate. He observed it to be very effective in lowering the blood pressure in both experimental and clinical cases. Recently the effect of benzyl benzoate and benzyl acetate upon blood pressure has been studied thoroughly by Gruber[8] upon dogs, cats and rabbits, and by Gruber and Shackelford[9] upon man. The former observer noted that benzyl benzoate, benzyl acetate, and benzyl alcohol did not lower blood pressure in dogs when given by stomach tube, even in amounts two hundred and ten times the customary dose for man. When given intravenously, these substances were noted to increase blood pressure in 8 per cent of the experiments and to decrease it in 90 per cent. The heart was the first portion of the cardiovascular system to be affected. The benzyl compounds in small doses decreased its volume, whereas large doses caused dilation. Like Musser,[10] and Allen and Sherrill,[11] Gruber and Shackelford[9] found benzyl benzoate to have no noticeable effect upon the blood pressure in their clinical cases of arterial hypertension.

Inasmuch as all the benzyl esters thus far studied, benzoate, acetate, mandelate, succinate, cinnamate, stearate, cyanide, salicylate, acetyl salicylate, phenolate, fumarate, para-amino-benzoate and monobenzyl barbiturate are

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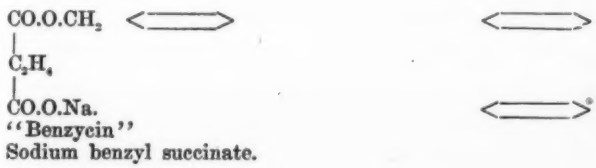
practically insoluble in water, and therefore, only slightly, if at all, soluble in the blood serum, it seems to us that intravenous administration of these drugs would be accompanied by undesirable side actions, the chief one among them being the formation of emboli. The quantity absorbed must also vary in proportion to the solubility of each drug so used. Recently there have been put on the market two double salts of the benzyl esters, which are soluble in water, "sodium benzyl succinate, benzycin, which contains about 39.5 per cent benzyl radical" and "sodium dibenzyl phosphate, benzyphos, which contains about 60.7 per cent benzyl radical."



Thus far I have been unable to find in the literature any report on the use of these preparations, nor have they, as far as I know, been accepted by the council on Pharmacy and Chemistry of the American Medical Association, for "New and Nonofficial Remedies."

According to the pamphlets sent to me by the manufacturers, these drugs have the same action as benzyl benzoate upon all smooth musculature and are indicated in all conditions in which benzyl benzoate would be used. They believe "benzycin" to be particularly valuable in "asthmatic conditions" and "benzyphos" to be of special use in relieving such conditions as "high blood pressure and uterine spasm."

This work was undertaken to determine the action of these drugs upon the respiratory and cardiovascular systems.

METHOD

The experimental animals used were dogs, cats, rabbits, terrapins, and frogs. The dogs were anesthetized with ether by inhalation or with paraldehyde (1.8 c.c. per kilo, per os.). The cats and rabbits were anesthetized with ether by inhalation or with urethane 2 gm. per kilo body weight by stomach. After anesthetization the animals were tracheotomized.

In the dogs, the injections were made directly into the exposed femoral vein. In the cats, the drugs were injected into the external jugular vein and also intraperitoneally, while in the rabbits they were injected into the lateral ear vein.

In all the experimental animals, except those in which perfusion was employed, the blood pressure was taken from a cannula inserted in the left carotid artery, and connected with a mercury manometer. A 2 per cent sodium citrate solution was used as an anticoagulant in those experiments in which the effects of the drugs were studied upon the respiratory systems, and in some of the experiments in which the drug was injected intraperitoneally. A sodium carbonate solution was used in almost all the remaining experiments. A chronograph marking 5, 10, 15, or 30 second intervals was

placed at the zero blood pressure level. Unless otherwise stated, all the writing points were placed one above the other.

The respirations in the dogs were recorded upon the kymograph surface by placing about the thorax a pneumograph connected with a Marey tambour. In the rabbits an S-shaped hook was fastened into the diaphragm through a median slit in the abdominal wall. By means of a ligature passing over pulleys, the S-shaped hook was connected to a writing lever so that the lever moved upward in expiration and downward in inspiration.

The organs of the abdomen were exposed through a mid-line incision. For plethysmographing a loop of intestine, or the kidney, metal oncometers were used connected either with piston recorders or with Gessell water tambours which marked the changes in volume upon the drum surface. The oncometers were applied with as little manipulation of the organs as possible and the abdomen then sewed up. A metal leg oncometer connected to a piston recorder was used in recording changes in the volume of the hind limb. In all instances, before the oncometer was applied the limb was disjointed at the ankle, so that the foot was not used in the plethysmographing oncometer. The limb and at least one other organ were plethysmographed simultaneously, and in many cases the volume changes of three organs were recorded at once, in addition to the recording of blood pressure.

After the establishment of artificial respiration, the changes in heart volume were recorded by placing a glass cardiometer about the heart, exposed by excising the ventral half of the thorax with the loss of as little blood as possible. Changes in blood pressure were recorded simultaneously. In a number of instances, records of both auricular and ventricular contractions were made simultaneously, with cardiographs[12] applied directly to the heart.

The hearts of dogs, cats, and rabbits were excised under ether anesthesia after exsanguination. These were perfused with Locke's solution, having a P_H of about 7.4, to which was added the defibrinated blood from the animal. In a number of dogs the kidney, loop of intestine, and hind limb were likewise perfused. The perfusion method was essentially the same as that used by Sherrington and Sowton.[13] The free end of the arterial cannula was connected to a short rubber tube leading from a reservoir containing the modified Locke's solution. This reservoir was kept in a water-bath at 38.5° C. and oxygenated by running oxygen through it. The perfusion pressure could be varied from 60 mm. of mercury to 100 mm. but was kept practically uniform throughout each experiment, by the rate of flow of oxygen into the perfusion reservoir. The excess oxygen was allowed to escape through a mercury valve which supported the desired pressure. During the escape of oxygen through the valve, the pressure would fall 4 mm. of mercury. This variation occurred about every five to ten seconds. Sodium benzyl succinate, or sodium dibenzyl phosphate dissolved in the perfusion mixture 0.1 gram per c.c., was injected into the perfusate near the arterial cannula against the perfusion fluid flow. Such injections had no effect upon the perfusion pressure as this was equalized by the escape of oxygen through the mercury valve.

The drops of perfusion fluid flowing from a glass cannula placed in the vein leading from the organ, were recorded on the kymograph by means of a receiving and recording tambour.

The hearts of rabbits and cats were perfused by inserting cannulas in the aorta near the organ; in the dogs, it was placed directly in the left coronary artery. The heart was immersed in the perfusion fluid in a glass cylinder. The excess fluid, as it escaped from the heart, was drained off and allowed to drop on a receiving tambour connected by means of rubber tubing to a Marey tambour, thus recording the rate of perfusion in drops. In a few cases, the heart was not surrounded by the fluid. The contraction of the heart was recorded on the kymograph by means of a light muscle lever connected by a ligature to the apex of the heart.

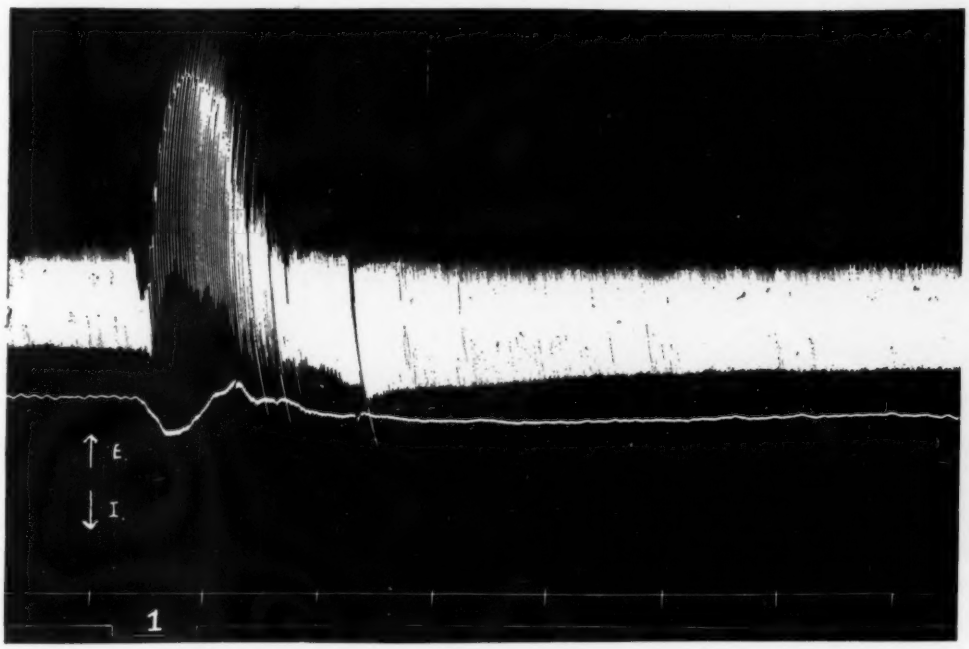


Fig. 1.—Rabbit, 1.7 kg., urethane anesthesia. In this and the following two records the top record is that of respiration, and below it in order, the blood pressure, time interval in thirty seconds and zero blood pressure, and the time of the injection. In this and the following two records the upstroke ↑ *E*, in the respiratory record, indicates expiration; the downstroke ↓ *I*, inspiration. At 1, 0.8 gram sodium dibenzyl phosphate dissolved in 4 c.c. of water was injected intravenously. Three and a half minutes after the end of this record, the blood pressure was 80 mm. mercury.

The effects of these drugs upon the frog's heart and the terrapin's heart were studied also. The animals were always pithed and the heart exposed in the usual manner. The contractions were recorded by connecting the right auricle and the apex of the ventricle to light muscle levers. The heart was kept moist by a continuous drip of Ringer's solution. When the drug was tested, the Ringer's solution in which the drug was dissolved was substituted for the original Ringer's solution.

RESULTS

Solubility.—One gram of sodium succinate is soluble in 2 c.c. and an equal quantity of sodium dibenzyl phosphate is soluble in 6 c.c. distilled

water at room temperature 24.6° C. A solution of calcium chloride (0.024 grams per 100 c.c., added to the sodium dibenzyl phosphate solution invariably results in the formation of a fine white flocculent precipitate. This fact was first noted in an attempt to dissolve the drug in Ringer's solution. This precipitate is probably an insoluble salt of calcium phosphate. After dissolving the drug in distilled water, the addition of Ringer's solution similarly caused a precipitate to be formed. We must then assume that this reaction takes place in the presence of the calcium of the blood stream and, therefore, intravenous administration of this drug is a questionable procedure in clinical therapy.

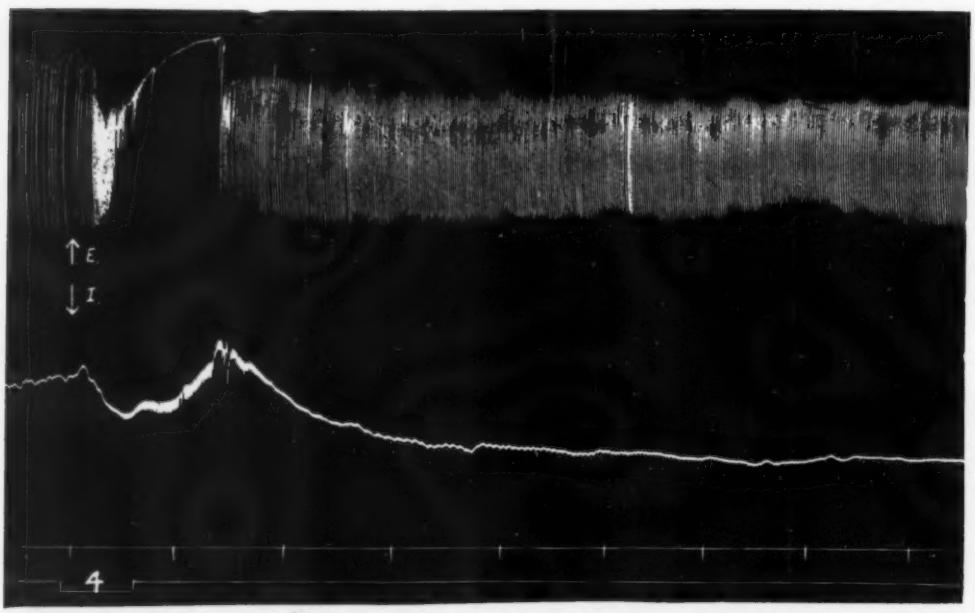


Fig. 2.—Rabbit, 1.8 kg., urethane anesthesia. Same as Fig. 1. At 4, 0.8 gram sodium dibenzyl phosphate dissolved in 4 c.c. of water was injected intravenously.

RESPIRATION

Like benzyl alcohol, acetate, and benzoate, sodium benzyl succinate and sodium dibenzyl phosphate when given intravenously cause in nearly all animals, an acceleration of the rate and a decrease in the depth of respiration. (Figs. 1, 2, and 3.) This is usually followed by a decrease in the rate with increased depth of respiration. Large doses produce paralysis of the respiratory center. (Figs. 2 and 3.) Whether the change in respiration is due to a direct stimulation of the respiratory center or a reflex stimulation due to the fall in blood pressure has not been determined. Mayer and Friedrich[14] found that a marked acceleration of respiration accompanies the fall in blood pressure, when amyl nitrite is given by inhalation. This they believed due to diminished blood flow through the respiratory center. Gesell, Capp, and Foote[15] noted an increase in respiratory rate when the blood pressure was lowered by hemorrhage, and concluded that the increase was due to the formation of acid within the center itself. It appears to me that there

may be a direct action upon the center with these benzyl compounds. In some cases the acceleration in respiration preceded the fall in blood pressure and in others, although the blood pressure did not fall appreciably and sometimes even rose, respiration was increased.

This acceleration in the respiratory rate is well shown in Figs. 1, 2, and 3, in which the upstroke of the lever indicates expiration and downstroke, inspiration. Figs. 1 and 3 were taken from the same animal, a rabbit weighing 1.7 kg. anesthetized with urethane. In Fig. 1, 0.8 gram sodium dibenzyl phosphate was administered intravenously at 1. There was an immediate increase in the rate and decrease in amplitude in respiration followed almost at once by slow, deep, forceful respirations which lasted about thirty seconds. Similar changes are observed in Fig. 3, in which 1 gram sodium dibenzyl

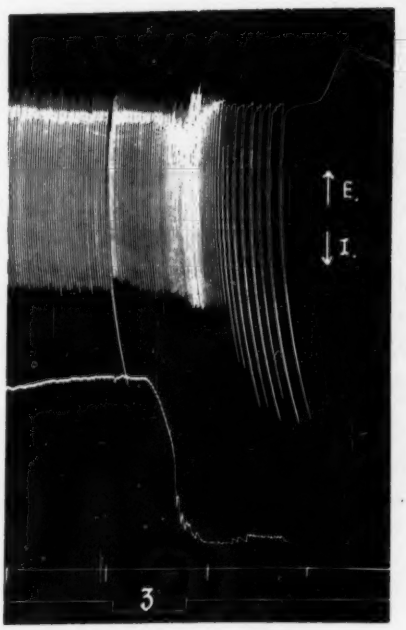


Fig. 3.—Rabbit, 1.7 kg., urethane anesthesia. Same animal as that used in Fig. 1. At 3, 1 gram sodium dibenzyl phosphate dissolved in distilled water was injected intravenously.

phosphate was injected. This injection caused permanent respiratory paralysis, probably of central origin. Upon stimulation of the phrenic nerve, after respiratory paralysis, the characteristic contraction of the diaphragm was still obtained.

A recovery from a temporary respiratory paralysis is observed in Fig. 2. This animal weighed 1.8 kg. Sodium benzyl succinate in one dose of 0.4 gram and one of 0.8 gram and sodium dibenzyl phosphate (0.4 gram) had been given intravenously before the injection seen in this figure. Following each of the preceding injections, there had been an acceleration in respiration, although the first injection caused a rise in blood pressure. In Fig. 2, 0.8 gram sodium dibenzyl phosphate was injected. There was total cessation of respiration for thirty seconds, accompanied by an asphyxial rise in arte-

rial blood pressure. A later injection of 1 gram of sodium dibenzyl phosphate caused permanent respiratory paralysis, the heart continuing to beat for some minutes.

BLOOD PRESSURE

Intravenous Administration.—The effects of sodium benzyl succinate and sodium dibenzyl phosphate upon blood pressure were not constant. In the main, there was a greater tendency toward a rise in blood pressure with smaller doses than with the larger ones. The effects that these drugs have upon arterial blood pressure may be seen in Figs. 1 to 5 and 8 to 10 inclusive and Tables I and II. In all the records except curves 5 and 9 fall in pressure is noted. With equal amounts by weight, this fall is more marked in every

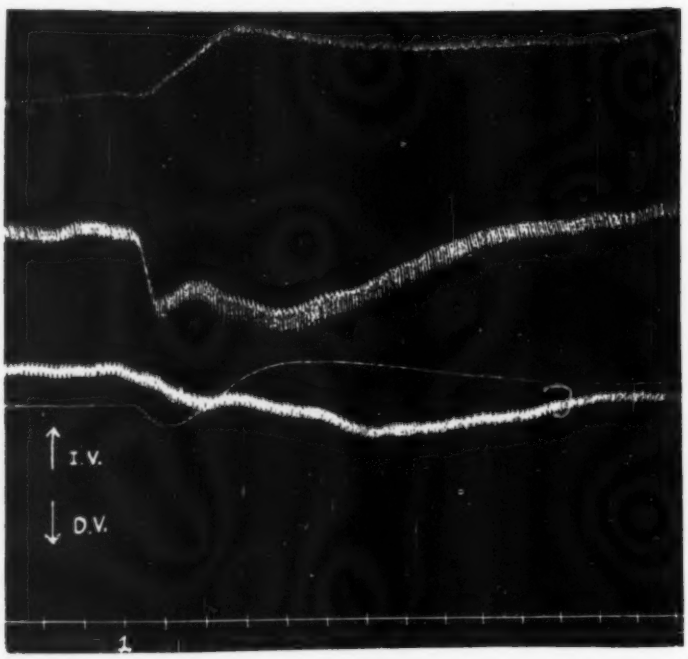


Fig. 4.—Dog, 17.7 kg., paraldehyde anesthesia. The top record is that of the plethysmographed intestine and below it in order, the blood pressure with mercury manometer, the volume of the limb, the kidney volume, the time in fifteen seconds and zero blood pressure, and time of the injection. The blood pressure writing point wrote 6 mm. in advance of the other three recorders. ↑ *I,V*, upstroke in the volume curves indicates increase in the volume and ↓ *D,V*, downstroke, decreased volume. At 1, 2 grams sodium dibenzyl phosphate dissolved in distilled water was injected intravenously.

case with sodium dibenzyl phosphate than with sodium benzyl succinate. In Table I, sodium benzyl succinate produced a fall in blood pressure in 20.3 per cent and a fall followed by a rise in 12.0 per cent of the injections. A rise was observed in 67.7 per cent of the cases. The fall in pressure varied from 3.2 to 35.5 per cent with an average of 14.7 per cent and the rise from 3.5 to 31.4 with an average of 13.0 per cent. Quite the opposite results were obtained with sodium dibenzyl phosphate. Fifty per cent of the injections produced a fall, 14.7 per cent a fall followed by a rise and only 35.3 per cent of the injections a rise in blood pressure. The percentile fall

in blood pressure varied from 8.0 to 66.6 with an average for the entire number of readings of 32.1 per cent. (Two readings are not included, as in these the blood pressure fell to zero and the animals died.) The percentile rise varied from 4.6 to 82.8 with an average rise of 17.3 per cent. See Table I. Somewhat similar results were obtained with rabbits and cats in which much larger doses were used. See Table II. In all the cases recorded in Tables I and II the increase in blood pressure following the injection of sodium benzyl

TABLE I

A SUMMARY OF THE RESULTS OBTAINED IN DOGS, UPON THE INTRAVENOUS INJECTION OF SODIUM BENZYL SUCCINATE AND SODIUM DIBENZYL PHOSPHATE. THE BLOOD PRESSURE READINGS ARE GIVEN IN MM. OF MERCURY

GRAMS SODIUM BENZYL SUCCINATE PER KILO. BODY WEIGHT	BLOOD PRESSURE BEFORE INJECTION	BLOOD PRESSURE AFTER INJECTION	FALL IN BLOOD PRESSURE IN PER CENT	RISE IN BLOOD PRESSURE IN PER CENT	GRAMS SODIUM DIBENZYL PHOSPHATE PER KILO. BODY WEIGHT	BLOOD PRESSURE BEFORE INJECTION	BLOOD PRESSURE AFTER INJECTION	FALL IN BLOOD PRESSURE IN PER CENT	RISE IN BLOOD PRESSURE IN PER CENT
0.02	92	86- 94	6.5		0.025	120	72	40.0	
0.055	92	88	4.3		0.04	143	124	13.3	
0.08	144	136-160	5.5		0.055	104	90	13.4	
0.11	96	66	3.2		0.055	106	90-110	15.1	
0.11	110	96	12.7		0.1	102	94-116	8.0	
0.13	102	84	17.6		0.11	170	94	44.7	
0.25	150	140	6.6		0.11	110	80	27.2	
0.25	150	140	6.6		0.11	102	72	29.4	
0.4	90	58	35.5		0.11	170	130-180	23.5	
0.4	88	62-100	29.5		0.11	160	104-176	35.0	
0.4	90	60- 99	33.3		0.13	152	104	31.5	
0.015	132	138		4.5	0.13	92	80	13.0	
0.03	62	76		22.6	0.15	160	0		
0.03	144	152		5.5	0.2	162	126	22.2	
0.03	60	78		30.0	0.2	120	40	66.6	
0.04	80	94		17.5	0.2	82	50	39.0	
0.04	130	142		9.2	0.2	96	58	39.6	
0.04	136	146		7.3	0.2	106	76-124	38.3	
0.05	78	95		21.8	0.22	100	45	55.0	
0.05	112	122		9.0	0.22	98	56	42.7	
0.055	86	94		9.3	0.25	154	84	45.4	
0.055	78	92		18.0	0.3	110	0		
0.055	70	92		31.4	0.015	128	134		4.6
0.06	110	126		14.5	0.02	122	136		11.4
0.077	135	150		11.1	0.03	150	163		8.6
0.1	94	106		12.7	0.03	100	110		10.0
0.11	90	100		11.1	0.03	90	100		11.1
0.12	133	142		6.7	0.03	100	104		4.0
0.2	88	102		12.5	0.04	132	147		11.3
0.2	152	164		7.9	0.055	98	110		12.2
0.22	188	204		8.4	0.06	126	144		14.3
0.22	168	174		3.5	0.08	80	98		22.5
0.23	142	168		18.3	0.14	58	106		82.8
0.25	146	156		6.8	0.25	140	160		14.3
Average fall in blood pressure in per cent			14.7					32.1	
Average rise in blood pressure in per cent				13.0					17.3

TABLE II

A SUMMARY OF THE RESULTS OBTAINED IN RABBITS AND CATS UPON THE INTRAVENOUS INJECTION OF SODIUM BENZYL SUCCINATE AND SODIUM DIBENZYL PHOSPHATE. THE BLOOD PRESSURE READINGS ARE GIVEN IN MM. OF MERCURY

GRAMS SODIUM BENZYL SUCCINATE PER KILO. BODY WEIGHT	BLOOD PRESSURE BEFORE INJECTION	BLOOD PRESSURE AFTER INJECTION	FALL IN BLOOD PRESSURE IN PER CENT	RISE IN BLOOD PRESSURE IN PER CENT	GRAMS SODIUM DIBENZYL PHOSPHATE PER KILO. BODY WEIGHT	BLOOD PRESSURE BEFORE INJECTION	BLOOD PRESSURE AFTER INJECTION	FALL IN BLOOD PRESSURE IN PER CENT	RISE IN BLOOD PRESSURE IN PER CENT
0.07	100	100			0.2	64	46	28.1	
0.25	96	(108-94)	2.6	12.5	0.2	68	37	45.6	
0.25	92	85	8.6		0.24	80	60	25.0	
0.25	86	96		11.6	0.25	80	84		5.0
0.25	100	96	4.0		0.25	77	84		9.1
0.5	92	36	61.0		0.37	68	20	70.7	
0.5	85	78-95	8.2	11.8	0.5	83	72	13.3	
0.6	82	94-38	53.7	14.7	0.5	72	(86-0)		19.5
0.075*	80	110		37.5	0.6	80	0		
0.075*	78	104		33.3	0.075*	92	70	23.9	
0.133*	90	116		28.8	0.133*	102	82-126	19.6	
0.333*	104	116-98-126	5.8	33.3	0.133*	104	78	25.0	
					0.33*	104	70	32.7	

*Cats under urethane.

succinate and sodium dibenzyl phosphate was a gradual one. In one curarized dog 0.14 gram per kilogram body weight of sodium dibenzyl phosphate, injected intravenously, increased the blood pressure in six minutes from 58 to 106 mm. of mercury, or an increase of 82.8 per cent. The heart rate increased simultaneously from 162 to 248 beats per minute.

Figs. 5 and 9 are presented to show the type of rise in blood pressure usually seen after the injection of these drugs. In Fig. 5, 4 grams sodium benzyl succinate, dissolved in distilled water, were injected intravenously in a dog weighing 17.7 kg. (0.22 gram per kilogram body weight). As a result of the injection the blood pressure was increased from 188 to 206 mm. of mercury, an increase of 8.4 per cent. In Fig. 4, 2 grams sodium dibenzyl phosphate was administered intravenously in the same animal before Fig. 5 was obtained. In this instance the blood pressure fell to 130 mm., but later rose to 180 mm. of mercury. In Fig. 9, a cat weighing 3 kg. was given 0.2 gram sodium benzyl succinate intravenously. The blood pressure increased from 78 to 99 mm. of mercury, or an increase of 27 per cent and the heart rate increased from 164 to 196 beats per minute, an increase of 32 beats. The blood pressure after nine minutes was still 90 mm. of mercury. Four-tenths of a gram of sodium benzyl succinate was later injected in this animal in a similar manner and the blood pressure rose this time from 88 to 116 mm. of mercury, an increase of 32 per cent. The heart rate was again accelerated, increasing from 184 to 204 beats, or 20 beats per minute.

A rise in blood pressure, following a fall, is observed in Figs. 1 and 2. In these, however, we attribute the rise in blood pressure not to the drug

injected, but to the asphyxia caused by the embarrassment of the respiratory mechanism as a result of the injection.

From the curves presented and from the collected data in Tables I and II, it will be seen that sodium dibenzyl phosphate, when given intravenously, appears to have a greater depressor action (depression of blood pressure) than does sodium benzyl succinate similarly administered. The dose appears to play an important part in the effect produced. Large doses cause a fall more often than do smaller ones and vice versa.

Intraperitoneal Administration.—The results obtained upon injecting sodium benzyl succinate and sodium dibenzyl phosphate intraperitoneally are interesting. Eleven cats under urethane anesthesia were employed. Twenty-four

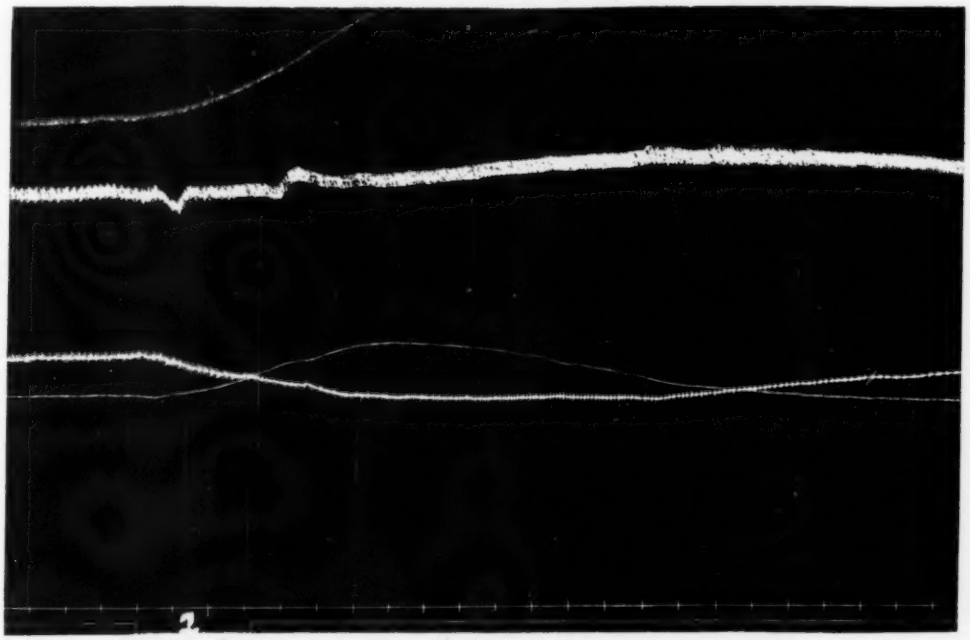


Fig. 5.—Continuation of Fig. 4. At 2, 4 grams sodium benzyl succinate dissolved in distilled water was injected intravenously.

injections were made, eighteen of which were with 0.5 to 1 gram of sodium benzyl succinate dissolved in 2.5 c.c. distilled water, and six with a similar quantity of sodium dibenzyl phosphate dissolved in 5 c.c. distilled water. In only a few cases 0.5 gram was used. Figs. 6 and 7 are typical of the results obtained from the sodium benzyl succinate injections. In all instances, within fifteen seconds after the injection, there was a sudden rise* in blood pressure varying from 10 to 75 per cent accompanied by dilation of the pupil. This rise was followed either by a gradual return to the control level as in Fig. 6 or after the return, by a second rise developing slowly and then gradually returning to the control level, as in Fig. 7. In a few instances the blood pressure

*Instead of the sudden rise in blood pressure in 8 rabbits similarly tested a sudden fall was observed in some cases as much as 40 mm. Hg.; otherwise, they responded in much the same way as cats.

dropped below the control level after the rise as was always the case following the sodium dibenzyl phosphate injections.

In Fig. 6, a cat weighing 3 kg. was anesthetized with urethane. After

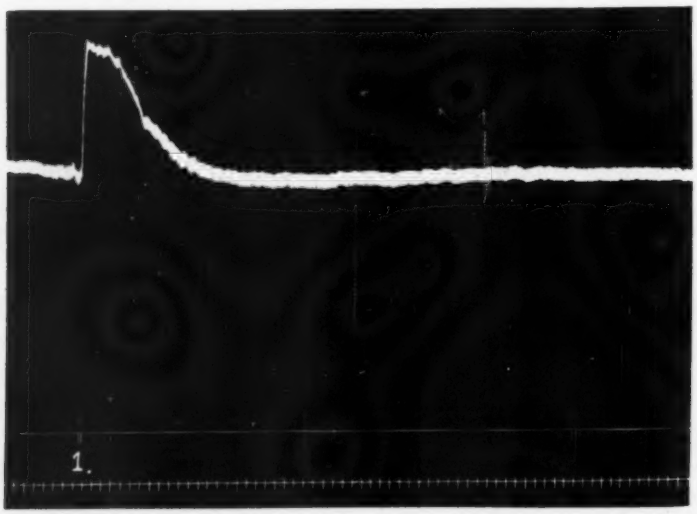


Fig. 6.—Cat, 3 kg., urethane anesthesia. In this and the following three records the top record is that of blood pressure in mm. of mercury, and the bottom record is the time interval in fifteen second intervals. The middle record is the point of injection and zero blood pressure. At 1, 0.5 gram sodium benzyl succinate dissolved in 2 c.c. of distilled water was injected intraperitoneally.

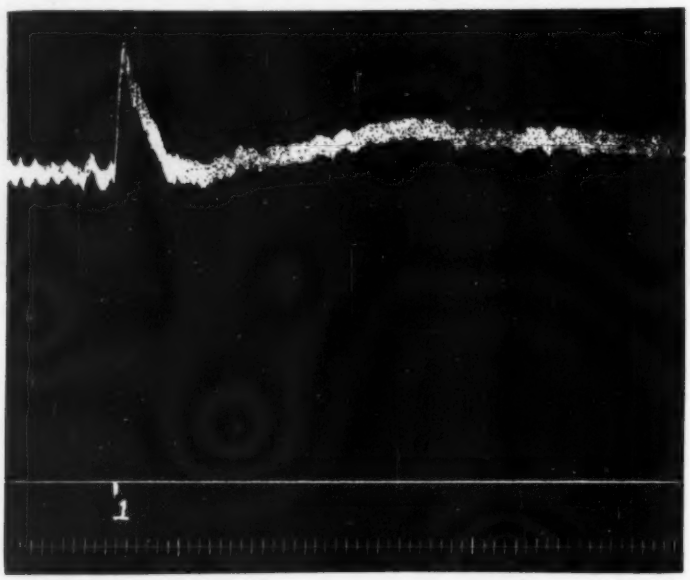


Fig. 7.—Cat, 2 kg., urethane anesthesia. Same as Fig. 6. At 1, 1 gram sodium benzyl succinate dissolved in 2.5 c.c. of distilled water was injected intraperitoneally.

the control level of blood pressure was well established, 0.5 gm. sodium benzyl succinate dissolved in 2 c.c. distilled water was injected intraperitoneally at 1. Approximately fifteen seconds after the injection the blood pressure suddenly increased from 114 to 176 mm. of mercury, an increase of 50 per

cent. This was later followed by a decrease in pressure to 108 mm. of mercury. In Fig. 7, 1 gm. sodium benzyl succinate dissolved in 2.5 c.c. distilled water was injected at 1. The blood pressure suddenly increased from 120 to 170 mm. of mercury, an increase of 43 per cent. This was followed by the typical gradual rise, the maximum being 140 mm. of mercury, or 16.6 per cent. After this curve was made, the circulation to both adrenal glands was clamped off and the injection repeated. Again, a typical rise in pressure resulted, though to a lesser degree. Whether the decrease was due to failure of adrenin to reach the circulation cannot be definitely stated, since each

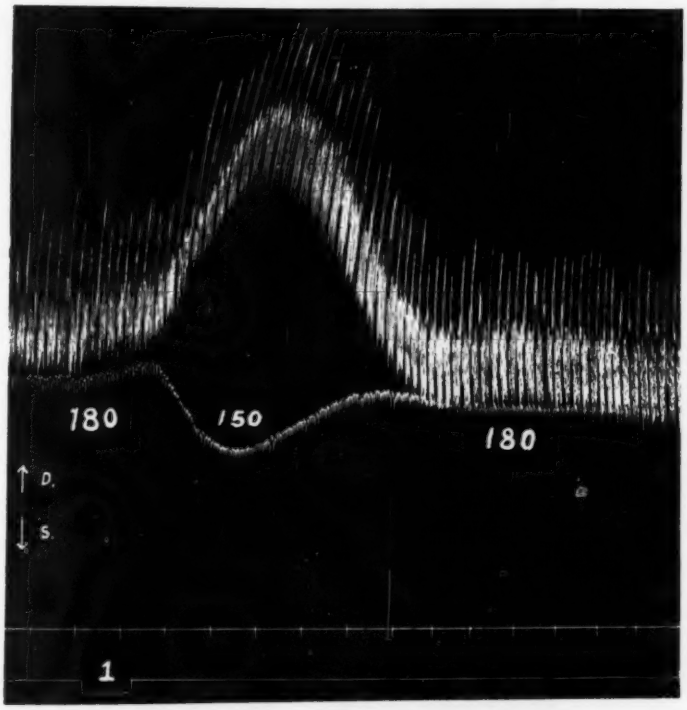


Fig. 8.—Dog, 18.6 kg., paraldehyde anesthesia. Artificial respiration. The top record is that of the plethysmographed heart; below it the blood pressure with mercury manometer. The bottom record is that of the time of injection, and above it, that of zero blood pressure and the time interval in ten second periods. In the cardiac record the upstroke ↑ *D*, is that of diastole and increased volume, downstroke ↓ *S*, is that of systole and decreased volume. At 1, an intravenous injection of 1.8 grams sodium benzyl succinate dissolved in 8 c.c. of Ringer's solution.

successive injection of a series is followed by a smaller increase in blood pressure than the injection before it.

We can only guess at the cause of the sudden rise in pressure, but we believe it is due to the concentrated sodium benzyl succinate solution becoming distributed in the splanchnic area causing either a powerful local vasoconstriction or reflexly exciting the sympathetic system into activity and simultaneously arousing the secretion of adrenalin. The latter assumption is further supported by the simultaneous dilation of the pupil. This, as near as we could determine, did not occur after cutting of the superior cervical sympathetic nerve trunk and extirpation of both adrenal glands.

Fig. 9.—Cat, 3 kg., urethane anesthesia. Artificial respiration. The records from the top downward are those of the myocardiograph record of the auricles, blood pressure with mercury manometer, myocardiograph record of the ventricles, time of injection, and the time interval in fifteen seconds and zero blood pressure. The heart rate is also indicated in the record. In this and Fig. 10, the upstroke ↑ *D*, in the myocardiograph record indicates diastole, the downstroke ↓ *S*, systole. At 1, 2 grams sodiumbenzyl succinate were injected intravenously.

ACTION UPON THE VOLUME OF THE INTESTINE, KIDNEY AND LIMB

In previous communications[8] on the effect of benzyl alcohol and its esters, benzyl benzoate, and benzyl acetate upon the cardiovascular system, the author noted a rise in blood pressure in 8 per cent of the experiments. He noted an acceleration of the heart rate and also vasoconstriction of the intestinal vessels after the injection in those cases in which the blood pressure was increased. At that time he advanced the hypothesis that the rise in pressure was due probably to two factors, increased volume output by the heart per unit of time and overcompensation of the vasomotor center. (The intestinal vessels, being more sensitive to the benzyl derivative, dilate and the cutaneous vessels, being less sensitive, constrict with the latter effect

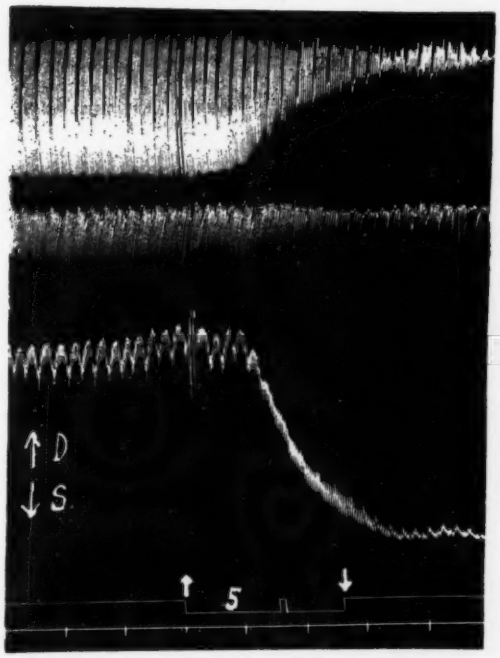


Fig. 10.—Dog, 10 kg., paraldehyde anesthesia. Artificial respiration. Same as Fig. 9, except that the record of the time of the injection is above that of the time interval. At 5, 3 grams sodium dibenzyl phosphate were injected intravenously.

predominating.) Experiments performed upon dogs and cats in which the intestine, kidney, and limb were plethysmographed, confirm this hypothesis.

In Fig. 4, a dog weighing 17.7 kg. was anesthetized with paraldehyde. At 1, 2 gm. sodium dibenzyl phosphate was administered. This caused an increase in the volume of the intestine, increase in kidney after a temporary passive decrease and a decrease in the limb volumes. The blood pressure which was 170 mm. of mercury dropped to 130 mm. but later rose to 180 mm. In all instances the vasomotor response was always more marked with sodium dibenzyl phosphate than with sodium benzyl succinate.

Fig. 5 was obtained from the same animal. At 2, 4 gm. sodium benzyl succinate was injected into the femoral vein. There quickly resulted a marked increase in the volume of the intestine. The kidney volume also in-

creased, the limb volume simultaneously decreasing. The blood pressure increased from 188 to 206 mm. of mercury and the heart rate from 160 to 204 beats per minute. In this instance the dilation of the intestine was so great as to cause its recording lever to leave the drum's surface.

CARDIAC EFFECT

Intact Heart.—The results already presented show that sodium benzyl succinate and sodium dibenzyl phosphate like benzyl benzoate and benzyl acetate can increase the rate of heart beat. This stimulation appears only with small doses and more commonly after the administration of sodium benzyl succinate. When these drugs are given in large doses the heart is

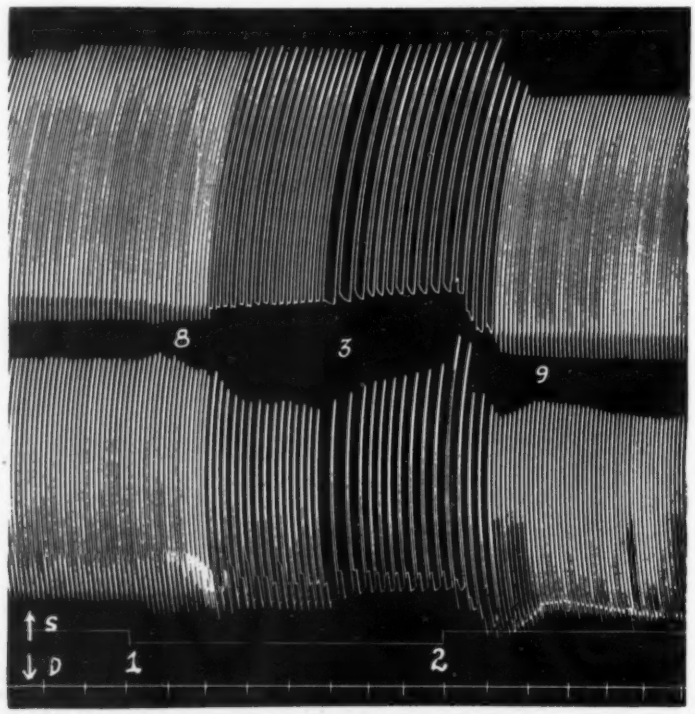


Fig. 11.—Terrapin, Chrysemys elegans. The top record is of contracting ventricle; below it in order are the records of the contracting right auricle and of the time that sodium benzyl succinate was dropped upon the heart, and the time interval in fifteen seconds. From points 1 to 2, 1 gram of sodium benzyl succinate dissolved in 2 c.c. of Ringer's solution was dropped on the heart. Upstroke ↑ *S*, indicates systole; the downstroke ↓ *D*, diastole. The heart rate is also indicated in fifteen second periods.

depressed. As further proof of this stimulatory action, experiments were performed upon intact and excised perfused hearts of dogs, cats, rabbits, and intact hearts of terrapins and frogs. Fig. 8 is a plethysmographic record of a dog's heart. The downstroke records systolic contraction, the upstroke, diastolic. At 1, 1.8 gram sodium benzyl succinate dissolved in 8 c.c. Ringer's solution was injected intravenously. The heart immediately dilated and the rate decreased 30 beats per minute. Within two minutes, however, the rate was normal and the volume of the heart was less than its original volume, i.e., the heart increased in tonus, and at the same time the excursion of the

lever showed the contractions to be more forceful. Such results were observed following most of the injection. Whenever the quantity injected was sufficient to produce a fall in blood pressure, the heart beat was slowed and the volume of the organ was increased. Smaller doses either had no effect or had a slight stimulatory action. Very large doses caused cessation of heart beat in which both auricles and ventricles stopped in diastole. Further confirmation of the accuracy of these findings was supplied by experiments in which cardiographs were applied to both auricles and ventricles. Figs. 9 and 10. In Fig. 9 (from a cat weighing 3 kg.), an increase in the force of muscular contraction, denoted by the increased excursions of levers attached

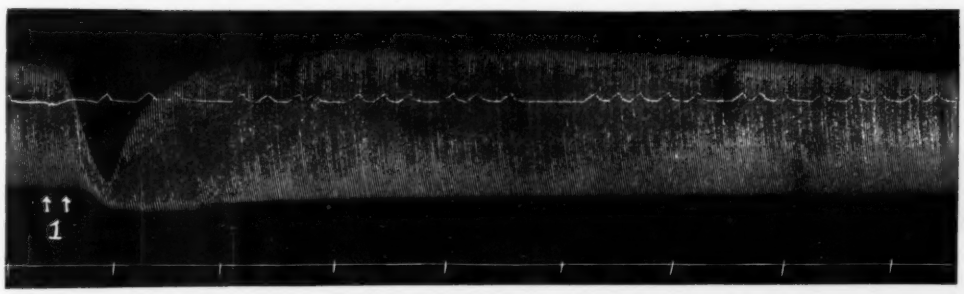


Fig. 12.—Isolated cat's heart perfused at 38.5° C. with Locke's solution, PH 7.4, to which was added about 15 c.c. of defibrinated autogenous blood per liter of fluid. The top record is the perfusion pressure in mm. of mercury; the middle record is that of the beating heart, and the bottom record that of the time interval in fifteen seconds and zero perfusion pressure. At 1, 0.1 gram sodium benzyl succinate dissolved in 2 c.c. of perfusion fluid was injected into the perfusion system near the aortic cannula.

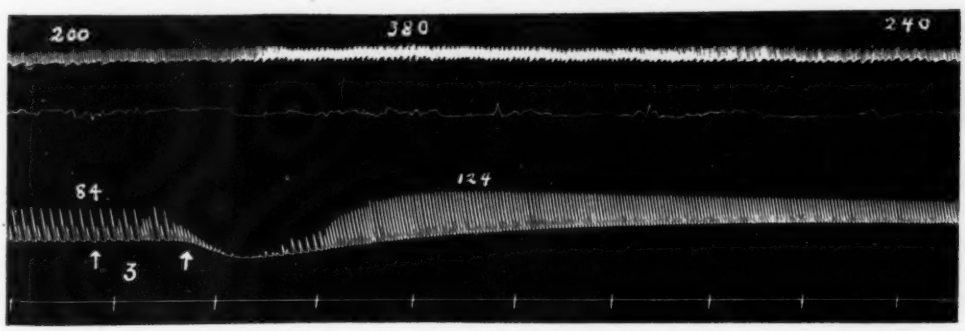


Fig. 13.—Same as Fig. 12. The top record is of the coronary flow in drops. At 3, 0.2 gram sodium benzyl succinate dissolved in 2 c.c. of blood Locke's solution was injected into the perfusate.

to both auricles and ventricles was noted after injecting 0.2 gm. sodium benzyl succinate. Here the effect appeared to be more marked in the auricles, but this was not always the case. In Fig. 10 is shown cardiac paralysis. In this instance 3 gm. sodium dibenzyl phosphate was injected intravenously in a dog weighing 10 kg. The heart dilated and finally ceased to beat, during which time the blood pressure dropped to zero.

Terrapin Heart.—In order to determine more accurately whether these drugs act as cardiac stimulants in small doses and cardiac depressants in large ones, they were tested upon intact terrapin and frog hearts by direct application and upon excised, perfused, mammalian hearts by injecting the

drug into the perfusate. It was found that dilute solutions of either drug when applied directly to the exposed terrapin heart had no effect. In a few cases the contractions appeared to become stronger but the results were so uncertain that no conclusions could be drawn. Strong solutions caused a decrease in the rate and in the extent of the excursion of contraction, which was always more marked in the auricles than in the ventricles. In Fig. 11, at 1, 2 c.c. of a 50 per cent solution of sodium benzyl succinate was dropped upon the heart, at 2 the benzyl solution was replaced by Ringer's solution. While exposed to the drug, the rate of contraction of the heart decreased from 8 to 3 beats per fifteen seconds. Upon replacing the benzyl derivative with Ringer's solution, the rate increased to 9 beats per fifteen seconds. A 16 per cent solution of sodium dibenzyl phosphate had a similar effect. In one case this strength of solution produced complete stoppage of the heart, from which it recovered after the drug was replaced by Ringer's solution.

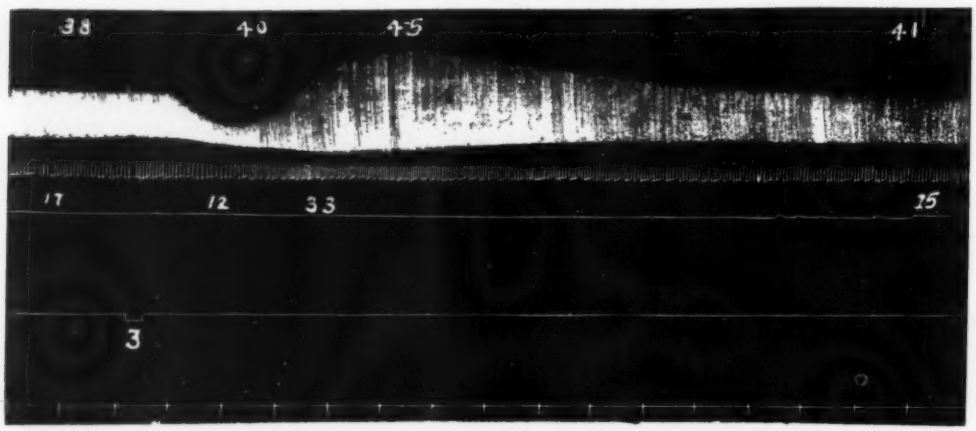


Fig. 14.—Excised rabbit's heart perfused with Locke's solution, PH 7.4, was modified by the addition of defibrinated autogenous rabbit's blood, 10 c.c. per liter of fluid. Records from above downward are: cardiac contraction, coronary flow in drops, perfusion pressure in mm. of mercury, time of injection, and time interval in fifteen seconds and zero pressure for perfusion. At 3, 0.1 gram sodium benzyl succinate dissolved in 1 c.c. of modified Locke's solution was injected into the perfusate.

The results upon the frog's heart were similar to those upon the terrapin's except in one respect. In the frog acceleration of heart beat is occasionally seen. This was always followed by a decrease in heart rate.

Excised Mammalian Heart.—Figs. 12, 13, 14, and 15 show the cardiac stimulating and depressing actions of these drugs upon the mammalian heart. In Fig. 12, a cat's heart was perfused, at 80 mm. mercury pressure, with warm Locke's solution with a P_H 7.4 containing autogenous defibrinated blood. At 1, 0.2 gm. sodium benzyl succinate dissolved in 2 c.c. Locke's solution was injected into the perfusion fluid near the perfusion cannula. The heart immediately dilated and the force of contraction diminished. Later the force of contraction increased, the rate remaining the same. After the perfusion pressure had been increased to 92 mm. of mercury, another injection was made in the same heart. Fig. 13. The heart immediately dilated and became so slow it almost ceased to contract. This was followed by the disappearance

of the pulsus alternans seen before this injection, and the rate and force of contraction were increased. The heart rate which was 84 beats per minute increased to 124 beats while the coronary flow simultaneously increased from 200 to 380 drops per minute. Since here the betterment in heart action corresponded with a marked dilation of the coronary arteries, the question arises: is the betterment in cardiac action due to bettered nutrition or have these drugs a direct stimulating action upon heart muscle? In Fig. 14, a rabbit's heart was perfused at a pressure of 88 mm. of mercury, with warm Locke's solution P_H 7.4 containing autogenous defibrinated blood. In this case a temporary constriction followed the injection. This constriction can also be accounted for by the dilation of the heart, thus retaining a greater portion of the blood within the heart muscle itself. Such constriction occurred in a number of hearts in which the solution appeared to be either fairly large in quantity or concentrated. A marked dilation followed. The rate of perfusion flow first decreased from 17 to 12 drops per fifteen seconds

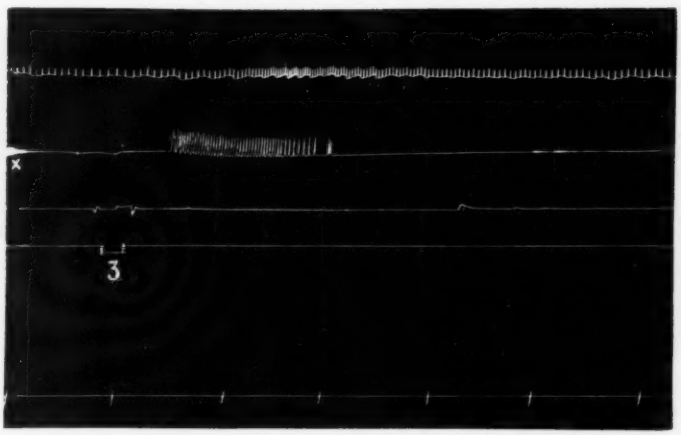


Fig. 15.—Excised rabbit's heart perfused with Locke's solution, PH 7.4 was modified by the addition of 10 c.c. of defibrinated autogenous blood to each liter of Locke's solution. The records from the top downward, show coronary flow in drops, cardiac contraction, perfusion pressure in mm. of mercury, time of injection of drug into the perfusate and time in fifteen seconds and zero perfusion pressure. *x*, vibration lever. At 3, 0.1 gram sodium benzyl succinate dissolved in 1 c.c. modified Locke's solution, was injected into the perfusate.

and then increased to 33 drops. The heart rate accelerated from 38 before the injection to 45 after. As in other records the decreased force of muscular contraction was accompanied by relaxation of muscle, i.e., dilatation. This increased coronary flow may be one of the factors concerned in the bettering of cardiac force but such results as those shown in Fig. 15, lead the author to believe that these drugs also have a stimulating action. In this experiment a rabbit's heart was perfused at 80 mm. of mercury pressure with warm Locke's solution P_H 7.4 to which was added defibrinated blood from the rabbit. At 3, 0.1 gm. sodium benzyl succinate dissolved in 1 c.c. Locke's solution containing blood, was injected into the perfusion system. Although the heart had stopped beating for some minutes after the injection it began to beat rapidly before any coronary dilation took place and ceased before the coronary flow had returned to its former rate. The rate of coronary flow in this case increased from 15 to 42 drops per fifteen seconds. In all cases so-

dium dibenzyl phosphate was found to produce greater dilation of the coronaries than did sodium benzyl succinate and it was also more toxic to cardiac muscle. Sometimes it caused cardiac acceleration, but in many instances, while the heart was still beating vigorously, it produced immediate and sometimes permanent cessation. Frequently the heart became arhythmic after its injection. In some instances there appeared to be established an A-V block and in others pulsus bigeminus, trigeminus, etc., and pulsus alternans.

In no case was the stimulating effect of these drugs equal to that produced by epinephrin in the same heart.

VASOMOTOR ACTION

Usually sodium benzyl succinate and sodium dibenzyl phosphate produced dilatation of the coronary arteries, when injected into the perfusate. This was demonstrated upon dogs, cats, and rabbits and occurred in both active and quiescent organs. A total of twenty injections of sodium benzyl succinate, varying in quantity from 0.1 to 0.5 gm., were made. An increase

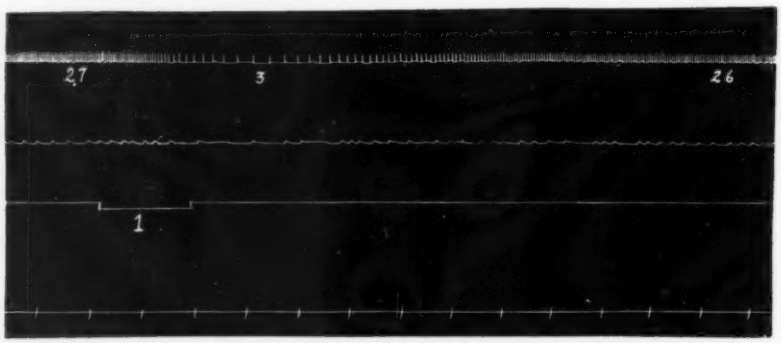


Fig. 16.—Cat, 3 kg., the animal was anesthetized with ether and exsanguinated. Right posterior limb was perfused with Locke's solution, PH 7.4, modified by the addition of defibrinated autogenous blood. The top record gives perfusion rate of flow in drops from the gracilis, adductor femoris, and semimembranosus muscles, and below it, the perfusion pressure in mm. of mercury. The bottom record shows time interval in fifteen seconds and zero pressure for the perfusion fluid, and above it the time of the injection. At 1, 0.025 gram sodium benzyl succinate dissolved in 0.25 c.c. modified by Locke's solution was injected into the perfusate.

in the rate of flow in drops from the heart was noted in the small doses and a decrease with large ones. Eighteen injections of sodium dibenzyl phosphate were made. In these a marked increase in rate of coronary flow was noted seventeen times. In one instance a large dose, 0.3 gm., produced a decrease in the coronary rate. Since this change occurred in both active and inactive hearts, it cannot, therefore, be attributed to increased activity of the organ. See Figs. 13, 14, and 15.

The effect that these drugs may have upon the rate of perfusion flow was also studied in the limb, intestine, and kidney. In the excised limb the gracilis, adductor femoris and semimembranosus muscles were perfused through the ramus muscularis branch of the femoralis artery[16] at a pressure varying from 60 to 80 mm. of mercury but constant for each experiment. Twenty-one injections were made and in these vasoconstriction, decreased rate of flow of fluid from the limb, occurred eighteen times. In three instances the rate was increased. Fig. 16 shows the usual result obtained from the limb.

At 1, 0.025 gm. sodium benzyl succinate was injected into the perfusate near the perfusion cannula. The rate of flow in drops decreased from 27 to 3 drops per fifteen seconds, but soon returned to its original rate. Loops of intestine were perfused, and in these, fourteen injections were made. The results here were somewhat more variable than those of the limb, but in the main, confirmed our plethysmographic findings. Increased rate of flow in drops was noted after the injections of these drugs in nine cases and decreased rate of flow in five. In the latter instances the quantity injected was usually large, 0.2 to 0.4 gm. The kidneys of three cats were perfused. In these, six readings were made. In all these the rate of flow in drops from the venous cannula was doubled after the injection of the drug. These results confirm our plethysmographic findings upon this organ.

The decreased rate in perfusion flow following the injection of concentrated solutions, we believe can be attributed mainly to the increased alkalinity of the injected fluid when either of these drugs is added to the perfusate and the action is that of increased P_H rather than that of a specific action of the drug.

DISCUSSION

Our results with the water soluble benzyl derivatives, sodium benzyl succinate and sodium dibenzyl phosphate, confirm our former views,[8, 9] that the benzyl esters are extremely unreliable for reducing blood pressure. Over 67 per cent of the injections of the former and 35 per cent of the injections of the latter resulted in increased blood pressure. Benzyl alcohol, benzoate, and acetate produced rises in blood pressure in 8 per cent of the injections.[8] We feel, as did Mason and Pieck,[5] that the point of action of the drug in those cases in which a fall in blood pressure results, is not the vessel wall only but also the cardiac muscle. Although these drugs can stimulate cardiac muscle, they probably are mainly cardiac depressants. If a sufficient dose is given to produce a fall in blood pressure, the first effect upon the cardiovascular system appears to be slowing the rate and dilatation of the heart with weakened contraction. The respiratory center is always first to be affected, in all injections. Usually there is acceleration in rate preceding the paralysis. It succumbs readily to comparatively small doses and is affected long before the heart is involved. The heart frequently beats some minutes after cessation of respiration. Occasionally an asphyxial rise in blood pressure is noted after cessation of respiration, showing that the vasomotor center has not been paralyzed simultaneously with the respiratory center.

SUMMARY

1. Sodium benzyl succinate is soluble in distilled water and Ringer's solution at room temperature (24.6° C.) in the proportion of 1 gram of the salt to 2 c.c. water or Ringer's solution.

2. Sodium dibenzyl phosphate is soluble in distilled water at room temperature (24.6° C.) in the proportion of 1 gram of the salt to 6 c.c. water. The presence of a calcium salt causes an insoluble flocculent precipitate to be formed. It is, therefore, insoluble in Ringer's solution and in the blood.

3. Both drugs appear to stimulate the respiratory center temporarily, causing an increase in rate with decreased depth followed by a slowed rate

and increased depth. Large doses cause paralysis of the respiratory center.

4. These drugs were injected 68 times in dogs, 8 times in cats, and 17 times in rabbits. The number of injections in dogs were equally divided between sodium benzyl succinate and sodium dibenzyl phosphate. Over 67 per cent of the injections of the former drug and 35 per cent of the latter produced rises in blood pressure. These rises averaged 13 and 17.3 per cent respectively. The remaining number of injections caused either a fall in blood pressure or a fall followed by a rise. Similar results were obtained in cats and rabbits.

5. Intraperitoneal injection of sodium benzyl succinate in cats causes a prompt increase in blood pressure, dilation of the pupil, and acceleration in respiration. In some cases the rise amounted to as much as 75 per cent. These changes we believe to be due to reflex stimulation of the entire sympathetic system, respiratory center, and possibly also the adrenal glands. The sudden increase is usually followed by a slow, progressive prolonged rise in blood pressure. In a few cases a fall was noted instead. Sodium dibenzyl phosphate also produced a sudden rise in pressure but this was followed in every case by a fall in pressure.

6. Intravenous injection of these drugs causes the volume of the intestine and kidney to increase but that of the limb to decrease.

7. Plethysmograph and myocardiograph records of the heart show these drugs, when injected intravenously in large doses, to cause dilation of the heart, slowed rate and finally cardiac paralysis. Small doses appear to increase the tonus and force and rate of contraction.

8. Perfusion experiments on the excised heart demonstrate these drugs to be cardiac stimulants in small doses and cardiac depressants in larger ones. With small quantities the increase in force and rate of contraction is always preceded by a decreased rate and force of contraction. In no case was the stimulation equal to that produced by epinephrin in the same heart. Large doses cause either temporary or permanent cessation of cardiac contraction. In some cases pulsus alternans, bigeminus, trigeminus and heart block appeared after the injection.

9. Local application of these drugs to the terrapin's heart and frog's heart causes a decrease in the rate and force of contraction of the heart. In a few frogs, an acceleration was noted.

10. Both drugs cause dilation of the coronary artery in small doses and constriction in larger ones. They also usually produce dilation of the intestinal and kidney vessels but constriction of the muscle vessels. The vasoconstriction we believe to be due mainly to the increased P_H of the fluid injected and not to a specific action of the drug.

11. Sodium dibenzyl phosphate was found to be more toxic and consequently more depressant to all tissues tested than was sodium benzyl succinate.

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GLYCOLYSIS AT VARYING BLOOD-SUGAR LEVELS*

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THE phenomenon of the disappearance of the sugar from the blood in vitro has been studied repeatedly since Claude Bernard's time. Lepine[1] believed that the explanation of the nature of diabetes lay in the difference between normal and diabetic blood in this regard. He postulated the existence in normal blood of an enzyme causing the glycolysis; the absence or decrease of this enzyme in diabetic blood should account for the lessened rate or complete inhibition of the glycolysis. Many others have observed this lessened glycolysis in diabetic blood specimens. Denis and Giles,[2] for example, working with newer methods reported two years ago, that glycolysis is much more active in normal blood specimens than in the blood of persons suffering from fairly severe diabetes. Thalhimer and Perry[3] also published similar suggestive results. If this could be established as always true, it might be possible to develop a method of investigating and evaluating borderline or doubtful cases of diabetes mellitus. The validity of the proposition has already been rejected by Eadie, Macleod, and Noble,[4] and Cajori and Crouter,[5] and by Tolstoi.[20] The data herewith presented (Table I) indicate that while, in general, blood rich in sugar or diabetic blood seems to lose its sugar at a much less rate than do normal blood specimens, there is no such invariable characteristic behavior of normal and diabetic blood in this respect as would permit a differential diagnosis by means of studies of glycolysis rates.

There has also been a discordance of opinion as to the behavior of glycolysis at varying levels of blood sugar. Denis and Giles[2] considered that "the amount of glycolysis obtained, bears no relation to the concentration of the blood sugar." Cajori and Crouter[5] came to the conclusion that the rate of the glycolysis varies directly with the amount of sugar present. Macleod[6] found that glycolysis proceeds at the same rate in normal and in hyperglycemic blood. (Splanchnic stimulation and adrenalin.) Tsubura's[7] experiments, to which further reference will be made presently lead to the opposite conclusion. We have approached the problem from a somewhat different angle and our results will contribute additional evidence upon this point.

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TABLE I

GLYCOLYSIS IN BLOOD TAKEN FROM PATIENTS IN FASTING STATE

CASE NO.	BLOOD SUGAR MG. PER 100 C.C.			MG. LOST	PERCENTAGE LOST
	IMMEDIATE		AFTER 24 HR. REFRIGERATION		
2000	D*	83	20	63	76
2084	D	266	143	123	46
2104	D	250	210	40	16
2121	D	250	226	24	9
2127	D	138	111	27	19
1297	D	97	54	43	44
2139	D	182	166	16	9
2150	D	266	266	0	0
2159	D	200	200	0	0
2170	D	235	222	13	5
2183	D	200	190	10	5
2181	D	222	174	58	21
2195	D	286	235	51	18
2252	D	266	250	16	6
2411	D	210	185	25	20
2322	D	256	154	102	46
2354	D	166	65	101	60
2383	D	200	200	0	0
2371	D	133	10	123	90
2373	D	333	222	111	33
2386	D	285	210	75	27
2381	D	266	211	55	20
2265	D	166	129	37	23
2077	D	111	57	54	48
1174	D	200	153	47	23
2117	D	190	163	27	14
2214	N	89	46	43	48
1685	N	166	95	61	42
2262	N	160	105	55	34
2066	R	100	50	50	50
2092	R	111	66	45	40
2038	R	87	39	48	55
2178	R	105	80	25	24
2203	R	97	65	32	33
2221	R	100	66	34	34
1824	R	111	111	0	0
2261	R	100	23	77	77
1596	R	111	58	53	46
2365	R	85	10	75	90
2310	R	89	20	69	77
2025	R	117	46	71	61
2415	R	89	18	71	79
2342	N	166	83	83	50
2343	N	111	54	57	51
2402	N	117	46	71	61
97	N	125	89	34	29
1868	N	105	26	79	75
1909	N	105	50	55	52
2073	N	105	36	69	65
204	N	111	67	34	39
R. H. B.	N	133	91	42	31
2143	N	111	100	11	9
2167	N	91	83	8	9
2157	N	111	54	57	51
2164	N	133	80	50	40
2174	N	105	100	5	5
2147	N	133	105	28	21
2182	N	100	91	9	9
2158	N	111	100	11	9
2210	N	95	41	44	56
2193	N	91	53	38	43
27	N	105	77	28	27
2227	N	111	71	40	36
2259	N	111	81	30	27

*D=Diabetes mellitus, R=Renal glycosuria, N=Nondiabetic.

METHODS

A. The percentage loss of glucose after twenty-four hours was used as the *index* of glycolysis. We have not undertaken to determine the velocity of the process, but base our observations on changes in the relative rate of blood sugar loss.

B. Sugar determinations were made by the Folin-Wu method.

C. The blood specimens were oxalated to prevent coagulation, except where otherwise indicated. This procedure is not free from criticism, since it has been pointed out that there is reason to believe that oxalate exercises an inhibiting influence upon glycolysis.[5, 6] There is evidence in the work of Denis and Giles,[2] in that of Cajori and Crouter,[5] and in our own, that the inhibiting effect of oxalate is peculiarly intense in diabetic blood. When this peculiar influence of oxalate upon diabetic blood specimens is explained, a further step toward understanding glycolysis will be taken. We enter into no discussion upon this point at this time. We believe that the variation in the degree of inhibition cannot be attributed to the influence of oxalate when the relation of the latter in the different experiments is held constant. For further evidence upon this point, we have divided some blood specimens into several parts to which citrate and varying quantities of oxalate were respectively added. These mixtures were kept under identical conditions and observations were made as to the blood sugar loss. It would appear (Table II) that no important rôle should be attributed to the oxalate in the experiments to be presented later.

TABLE II

GLYCOLYSIS RATES OF DIABETIC BLOOD WITH CITRATE AND WITH VARYING AMOUNTS OF OXALATE (CASE 1692)

DATE	ANTICOAGULANT	BLOOD SUGAR IMMEDIATE	BLOOD SUGAR AFTER 24 HR.	MG. LOST	PERCENTAGE LOST
1925					
1/20	100 mg. citrate per 10 c.c. blood	228	228	0	0
1/20	20 mg. oxalate per 10 c.c. blood	228	228	0	0
1/20	40 mg. oxalate per 10 c.c. blood	228	228	0	0
1/27	100 mg. citrate per 10 c.c. blood	141	111	30	21
1/27	20 mg. oxalate per 10 c.c. blood	141	117	24	17
1/27	40 mg. oxalate per 10 c.c. blood	141	125	16	11
2/12	100 mg. citrate per 10 c.c. blood	121	111	10	8
2/12	20 mg. oxalate per 10 c.c. blood	121	118	3	2
2/12	40 mg. oxalate per 10 c.c. blood	121	121	0	0

D. The blood specimens were kept in a refrigerator at a temperature of from 9° to 11° Centigrade rather than in an incubator or at room temperature. This was done to minimize the possible influence that bacterial growth might have as a factor in causing glycolysis. While it has been repeatedly suggested that the disappearance of sugar from blood in vitro may be due to, or be materially influenced by bacterial contamination, investigators who have been careful to control their work bacteriologically have failed to demonstrate that the variations in glycolysis found by them could be ascribed to bacterial action.[5, 6, 7, 8, 20] These variations occurred when controls indicated perfect asepsis. Aseptic precautions were therefore considered superfluous. Attention is further directed to the fact (to be developed later) that

glycolysis was, in general, less in the blood specimens richer in sugar; whereas, if bacteria were responsible for the disappearance of the sugar, we would find their growth to be more luxuriant and their influence greater and, hence, the glycolysis more intense in the blood richer in sugar.

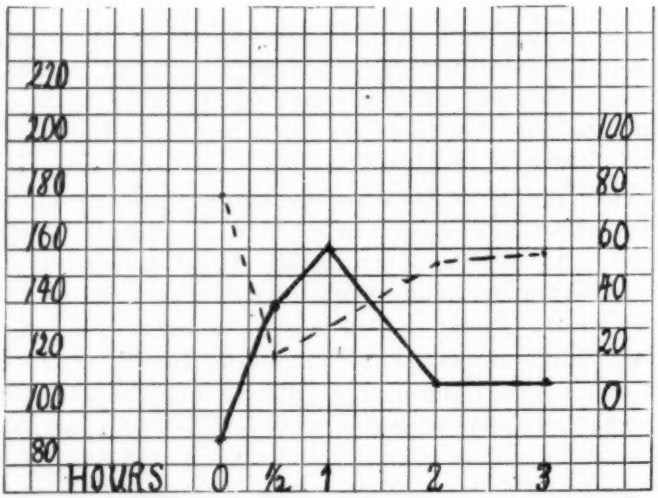

Chart 1.—Case No. 2415. Renal glycosuria. Glucose meal, 100 grams on fasting stomach. Typical relation between blood-sugar and glycolysis curves.

The solid line indicates blood sugar. Dotted line indicates percentage loss after standing 24 hours in refrigerator. Figures at right indicate percentage of blood-sugar loss. Figures at left indicate blood-sugar levels.

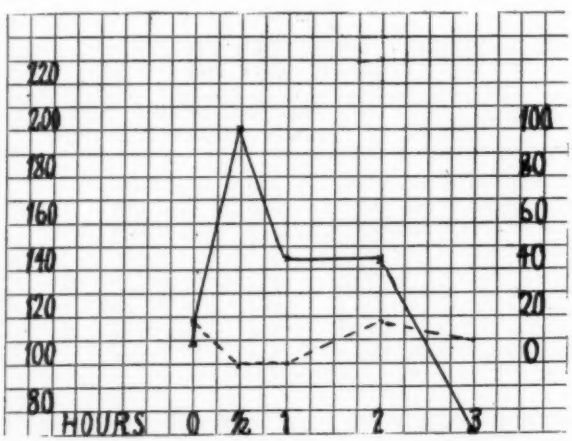

Chart 2.—Case No. 2359. Chronic nephritis. Arteriosclerosis. Hypotension. Glucose meal, 100 grams on fasting stomach. Marked rise in blood sugar and characteristic subsequent physiologic hypoglycemia. Characteristic glycolysis inhibition.

The solid line indicates blood sugar. Dotted line indicates percentage loss after standing 24 hours in refrigerator. Figures at left indicate blood-sugar levels. Figures at right indicate percentage of blood-sugar loss.

Most of our specimens were taken from patients in the fasting state (this was the case in all the specimens of Tables I and II). Occasionally, specimens were collected after a meal. The evidence suggested that alimentary hyperglycemia causes a slowing of the glycolysis rate in oxalated blood. The results are not invariable but, in general, where the blood sugar was not

TABLE III

GLYCOLYSIS IN BLOOD TAKEN BEFORE AND AFTER BREAKFAST (USUALLY COFFEE, MILK, SUGAR, PANCAKES AND SYRUP)

CASE NO.		BLOOD TAKEN BEFORE BREAKFAST BLOOD SUGAR MG. PER 100 C.C.				TIME AFTER MEAL IN HOURS	BLOOD TAKEN AFTER BREAKFAST BLOOD SUGAR MG. PER 100 C.C.			
		IMMEDIATE	AFTER 24 HR. REFRIGERATION	MG. LOST	% LOST		IMMEDIATE	AFTER 24 HR. REFRIGERATION	MG. LOST	% LOST
1596	R*	100	54	46	46	½	111	58	53	48
2292	R	100	44	56	56	½	118	71	47	39
2342	N	148	83	65	44	½	166	69	97	58
2371	D	133	20	113	85	½	250	200	50	20
2442	D	133	105	28	21	¾	333	250	83	25
2355	D	182	182	0	0	¾	222	222	0	0
2355	D	200	189	11	5.5	¾	235	230	5	2
2453	D	149	140	9	6	¾	190	183	7	4
E. P.	N	91	62	29	32	¾	105	80	25	24
R. T. L.	N	111	72	39	35	¾	91	77	14	15
I. I. L.	N	111	87	24	22	¾	117	83	34	29
2430	N	105	80	25	24	1¼	111	83	28	25
Miss D.	N	100	77	23	23	¾	105	88	17	16
Miss S.	N	105	83	22	21	¾	117	66	51	44
1596	R	100	54	46	46	½	111	58	53	48
2292	R	100	44	56	56	½	118	71	47	40
2343	N	105	66	39	37	½	125	103	20	16
2371	D	133	10	123	85	¾	250	200	50	20

*D=Diabetes mellitus, R=Renal glycosuria, N=Nondiabetic.

raised, the relative slowing of the glycolysis was not apparent. In Table III, the detailed results are given of blood specimens taken before and after a meal (usually pancakes, syrup, coffee, milk, and sugar). In striking contrast to the somewhat variable results quoted above are those obtained when hyperglycemia is produced by the ingestion of 100 grams of glucose, that is to say, during the course of the so-called blood-sugar curve or glucose tolerance test. In Table IV, there is seen in each case a slowing of the glycolysis rate with the rise of the blood-sugar level; as the hyperglycemia passes off, the glycolysis rate rises again. Charts 1, 2, 3, and 4 illustrate this point graphically. There is a divergence of the blood-sugar and glycolysis curves except in the case of Addison's disease (Chart 3), when no hyperglycemia occurred because of the increased tolerance for sugar. Chart 4 shows the low glycolysis rate usually found in diabetic blood, but even in this chart there is a divergence of the two curves. The most striking is Chart I where a scissor-effect is produced. This is the usual form of chart in normal individuals.

The slowing of the glycolysis with the rise of the blood sugar was put to the further experimental test by the intravenous infusion of glucose in two nondiabetic individuals. The results are similar to those where the glucose was administered by mouth. (See Table V.) In the third experiment exhibited in this table the peak of the glycemia was missed, because the blood specimen was taken too late and the inhibition of the glycolysis had passed off. It seems possible that if the blood had been taken earlier after the injection of the glucose solution, in both experiments on this patient, the results would have been more striking. Several intravenous infusions of glucose were given to a third patient, with chronic arthritis, and similar results were obtained. As the blood-sugar level rose, there was each time a fall

in the relative rate of the loss of blood sugar. Details are given in Table VI and one of the experiments is illustrated in Chart 5. In the first experiment there is a usual, but not striking, result. In the second experiment insulin

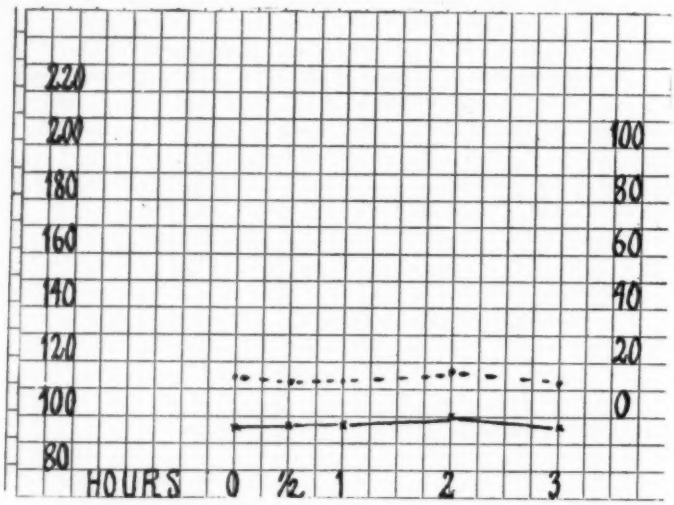

Chart 3.—Case No. 2471. Addison's disease. Increased tolerance for glucose. Glucose meal, 100 grams on fasting stomach. Flat blood-sugar curve. Flat glycolysis curve.

Solid line indicates blood sugar. Dotted line indicates percentage loss after standing 24 hours in refrigerator. Figures at left indicate blood-sugar levels. Figures at right indicate percentage of blood-sugar loss.

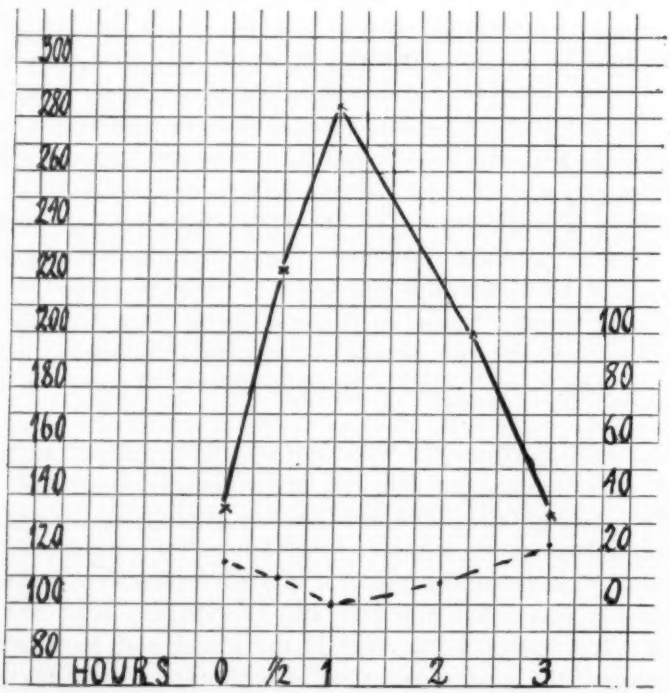

Chart 4.—Case No. 514. Mild diabetes mellitus. Glucose meal, 100 grams on fasting stomach. Low glycolysis rate throughout, the usual findings with diabetic bloods.

The solid line indicates blood sugar. Dotted line indicates percentage loss after standing 24 hours in refrigerator. Figures at left indicate blood-sugar levels. Figures at right indicate percentage of blood-sugar loss.

TABLE IV

GLYCOLYSIS IN BLOOD SPECIMENS TAKEN DURING BLOOD SUGAR CURVE AFTER MEAL OF 100 GRAMS OF GLUCOSE

CASE NO.		BLOOD TAKEN WITH RELATION TO MEAL	BLOOD SUGAR—MG. PER 100 C.C. IMMEDIATE	AFTER 24 HOUR REFRIGERATION	MG. LOST	PERCENTAGE LOST
2415	R*	Fasting	89	18	71	80
		½ hr. after	138	111	27	20
		1 hr. after	160	111	49	31
		2 hr. after	111	50	61	55
		3 hr. after	111	47	64	58
2310	R	Fasting	89	20	69	77
		½ hr. after	143	133	10	7
		1 hr. after	108	66	42	39
		2 hr. after	105	80	25	24
		3 hr. after	110	99	11	10
514	D	Fasting	133	111	22	16
		½ hr. after	222	200	22	10
		1 hr. after	285	285	0	0
		2 hr. after	200	182	18	9
		3 hr. after	133	105	28	21
2157	N	Fasting	80	53	27	34
		½ hr. after	114	50	64	56
		1 hr. after	80	77	3	4
		2 hr. after	83	80	8	10
		3 hr. after	83	20	63	76
2471	A	Fasting	95	71	24	25
		½ hr. after	100	80	20	20
		1 hr. after	100	100	0	0
		2 hr. after	100	80	20	20
		3 hr. after	100	74	26	26
2471	A	Fasting	95	80	15	16
		½ hr. after	95	83	12	13
		1 hr. after	95	83	12	13
		2 hr. after	100	83	17	17
		3 hr. after	95	83	12	13
2359	N	Fasting	111	95	16	14½
		½ hr. after	200	200	0	0
		1 hr. after	144	144	0	0
		2 hr. after	144	118	26	18
		3 hr. after	62	55	7	11

*D=Diabetes mellitus, R=Renal glycosuria, N=Nondiabetic.

TABLE V

GLYCOLYSIS FOLLOWING INTRAVENOUS INFUSION IN NONDIABETIC PATIENTS (INFUSION = 20 C.C. OF 50% GLUCOSE SOL. = 10 GRAMS.)

DATE	PATIENT NO.	BLOOD TAKEN WITH RELATION TO INFUSION	BLOOD SUGAR IMMEDIATE	MG. PER 100 C.C. AFTER 24 HR. REFRIGERATION	MG. LOST	PERCENTAGE LOST
1924						
10/23	T-6150	Before	80	66	14	17.5
		30 min. after	58	51	7	12
		75 min. after	100	66	34	34
10/21	T-4873	Before	83	67	16	18
		45 min. after	111	111	0	0
		60 min. atter	80	65	15	19
11/6	T-4873	Before	95	20	75	79
		45 min. after	105	20	85	81
		120 min. after	100	40	60	60

was given hypodermically at the time of the glucose infusion, and there was not a subsequent hyperglycemia, but, in spite of this, the blood-sugar and glycolysis curves exhibit their usual divergence. In the subsequent experiments stronger glucose solutions were used; a hyperglycemia was produced and, with it, a more striking inhibition of the glycolysis. In the fifth experiment on this patient there was an initial postprandial hyperglycemia, 166 mg., which was raised to 222 mg. by the infusion, and the relative glycolysis rates were respectively 7 per cent and 5 per cent. A marked hypoglycemia followed (40 mg.) due in part to the insulin and in part, no doubt, to the physiologic effect of the glucose infusion. With this marked hypoglycemia there was a rise in the relative glycolysis rate to 32 per cent.

These results upon normal and diabetic individuals were obtained before the reports of the experiments of Tsubura,[7] published in June, 1924, had

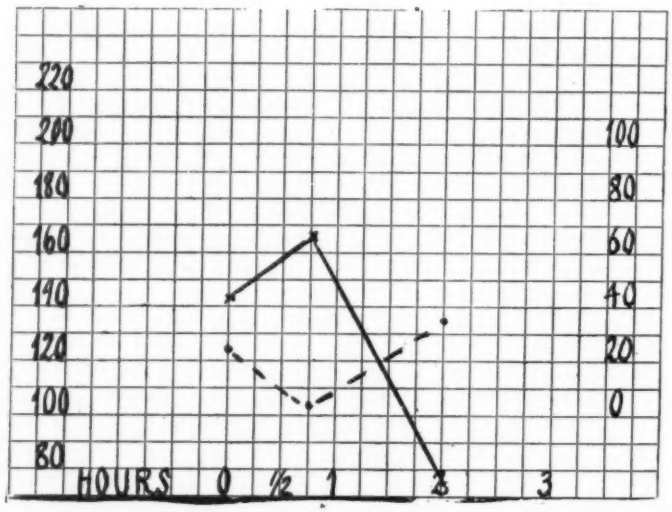

Chart 5.—Case No. 1876. Chronic arthritis. October 30, 1924. Intravenous infusion of 200 c.c. 20 per cent glucose. Insulin, 10 units hypodermically. Hyperglycemia and subsequent hypoglycemia. Typical relation between blood-sugar and glycolysis curves.

The solid line indicates blood sugar. Dotted line indicates percentage loss after standing 24 hours in refrigerator. Figures at left indicate blood-sugar levels. Figures at right indicate percentage of blood-sugar loss.

come to hand. His results upon the guinea pig blood are similar to those we have seen in human blood. He studied the glycolysis rate in defibrinated blood without the addition of any anticoagulant and compared the glycolysis of normal blood, (1) with blood fortified by the addition of glucose in vitro and, (2) with blood specimens of various hyperglycemic states. The latter were induced by adrenalin injections, by fourth ventricle punctures, by suffocation, by sodium chloride intravenous infusion, by caffeine, theobromin, and agurin injections, by restraint, and by hemorrhage. There was slight decrease in the glycolytic rate after the addition of glucose to the blood in vitro. When specimens of hyperglycemic blood were compared with normal blood fortified to the same sugar levels, it was found that there was evident glycolysis inhibition in the hyperglycemic states, except that following the injections of caffeine, theobromin, and agurin. The degree of in-

TABLE VI

GLYCOLYSIS IN BLOOD BEFORE AND AFTER GLUCOSE INFUSIONS WITH AND WITHOUT INSULIN IN A CASE OF CHRONIC ARTHRITIS

DATE 1924	GLUCOSE INFUSION	UNITS OF INSULIN		BLOOD SUGAR MG. PER 100 C.C.			
				BLOOD TAKEN BEFORE BREAKFAST	BLOOD TAKEN AFTER BREAKFAST	BLOOD TAKEN 45 MIN. AFTER GLUCOSE INFUSION	BLOOD TAKEN 2 HR. AFTER GLUCOSE INFUSION
I	200 c.c. of	0	Immediate	91		111	69
10/27	10% Sol.		After 24 hours	74		100	50
	(20 gm.)		Mg. lost	17		11	19
			Percentage lost	19		10	28
II	200 c.c. of	10	Immediate	83		83	57
10/28	10% Sol.		After 24 hours	62		77	50
	(20 gm.)		Mg. lost	21		6	7
			Percentage lost	35		7	12
III	200 c.c. of	10	Immediate		95	143	111
10/29	20% Sol.		After 24 hours		62	117	71
	(40 gm.)		Mg. lost		33	26	40
			Percentage lost		25	18	36
IV	200 c.c. of	10	Immediate		143	166	77
10/30	20% Sol.		After 24 hours		111	154	50
	(40 gm.)		Mg. lost		32	12	27
			Percentage lost		22	7	35
V	200 c.c. of		Immediate		166	222	40
10/31	20% Sol.		After 24 hours		154	210	27
	(40 gm.)		Mg. lost		12	12	13
			Percentage lost		7	5	32

TABLE VII

GLYCOLYSIS RATES OF BLOOD OF DIABETIC PATIENTS WITH AND WITHOUT INSULIN

CASE NO.	BLOOD SUGAR MG. PER 100 C.C.				INSULIN
	IMMEDIATE	AFTER 24 HR. REFRIGERATION	MG. LOST	PERCENTAGE LOST	
2373	333	222	111	33	No
2373	222	87	135	60	No
1951	181	174	7	4	Yes
1749	91	74	17	19	No
1749	166	154	12	7	Yes
2386	285	210	75	26	No
2381	266	211	55	21	No
2381	235	200	35	15	Yes
1692	182	133	49	27	Yes
2402	160	154	6	4	Yes
2402	149	140	9	6	Yes
2355	182	182	0	0	Yes
2355	200	189	11	5.5	Yes

hibition was in direct ratio to the level of the hyperglycemia; as the latter rose, the glycolysis rate fell, and as the hyperglycemia passed off, the glycolysis rate rose again.

It would appear that glycolysis inhibition is not affected by insulin per se. This is in accord with the experiments of Eadie, Macleod, and Noble,[4] of Mauriac and Aubertin,[9] and others[10, 11] who have shown that no glycolytic action is to be attributed to insulin. In this connection, too, we exhibit, in Table VII, some observations upon glycolysis rates in diabetic patients, some

of whom were taking insulin and others of whom were not taking insulin. These also show that no apparent effect is to be attributed to the use of insulin.

The subject of glycolysis is still a fruitful field for investigation. Different blood specimens lose their sugar at different rates and the same blood loses its sugar at different rates under different circumstances. Whether this phenomenon is due to the action of an enzyme is still to be proved. It has been proved that the glycolytic power resides in the formed elements of the blood;[6, 12, 19] once the blood is laked with water, the glycolysis ceases. In this connection, we have speculated upon a possible relation between the physiologic leucocytosis occurring after meals and the glycolysis. It is evident at once, however, that the explanation cannot lie in this quarter, for a leucocytosis should bring about an increased glycolysis, not a decreased glycolysis as does occur after a meal. Tolstoi[20] and also Thalhimer and Perry[3] could find no correlation between leucocyte counts and glycolysis. Nor is it at all certain that the same glycolysis occurs in vivo as in vitro. Claude Bernard, Lepine,[1] Chauveau,[13] and other older authorities found less sugar in venous than in arterial blood and considered, therefore, that glycolysis occurred in the tissues. Later, Chauveau and Kaufman[13] and recently Mauriac and Aubertin[9] showed that the glycolytic power of the tissues is no less in depancreatized animals than in normal ones. The theory of Winter and Smith,[14] that there exist several forms of blood sugar, has not found substantiation at the hands of other investigators.[15,16,17,18] If their theory were true, the several forms of blood sugar might behave differently with respect to the process of glycolysis. The blood sugar in the general circulation after a meal or after a glucose infusion may be of a different nature from that which is given off slowly from the glycogen store of the liver. The former sudden increment has not been stored and has not undergone the changes incidental to such storing. All of this is pure speculation.

CONCLUSIONS

The present report indicates:

1. Glycolysis proceeds at a lessened rate when the level of blood sugar is raised, not only by glucose (or other food) ingestion, but also by intravenous glucose infusion.

2. The characteristic behavior of glycolysis in diabetic blood specimens may be related to the quantitative level of the blood sugar rather than to any essential quality of the blood.

3. Administration of insulin does not affect the characteristic behavior of glycolysis either in diabetic or in nondiabetic blood.

4. No important rôle is to be attributed to oxalate in evaluating the inhibition of glycolysis.

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THERAPEUTIC DYES*

I. Mercurochrome-220, Soluble

By R. W. French, Washington, D. C.

WITH the increasing demand for mercurochrome-220, soluble, the fact that its manufacture is not protected by patents, and that products of doubtful merit have been marketed under that name, need for means of analysis and standardization has become apparent. Early in 1924, on the assumption that a fixed chemical composition should result in a fixed dye and dye content, spectrophotometric methods of analysis were adopted. This method of study has been applied to mercurochrome-220, soluble.

From the examination of a number of samples submitted by Edwin C. White, the discoverer, it has been found that the genuine product as prepared under his supervision is extremely uniform and readily permits of the application of such methods of control. The accuracy of determination, both qualitatively and quantitatively, is believed to be quite high.

Chemically mercurochrome-220, soluble, is the disodium salt of dibromohydroxymercury-fluorescein or mercurated dibromfluorescein, the mercury probably entering into para position to the linking oxygen.[1] Thus, it is chemically somewhat similar to eosin.

*From the Laboratory Service, Walter Reed General Hospital, Army Medical Center, Washington, D. C.
Received for publication, May 15, 1925.
[1]White, E. C.: Jour. Am. Chem. Soc., 1920, xlii, No. 11.

Spectrophotometrically dibromfluorescein has a distinct maximum of absorption at 504.8 mμ according to Formanek[2] while eosin, according to the same author, has a maximum at 516 mμ. A tentative specific transmissive index determined for dibromfluorescein at 504.8 mμ is 1.54 while that of eosin at 516 mμ is 1.2155. Mercurochrome-220, soluble shows a distinct maximum of absorption at 512 mμ with a specific transmissive index from the mean of 13 samples of 0.804.

From this it will be noted that the addition of mercury to the dibromfluorescein has shifted the maximum of absorption toward the longer wave lengths of the spectrum 7.2 mμ and that there is relatively less color in the resultant chemical as evidenced by the lower value of the specific transmissive index.

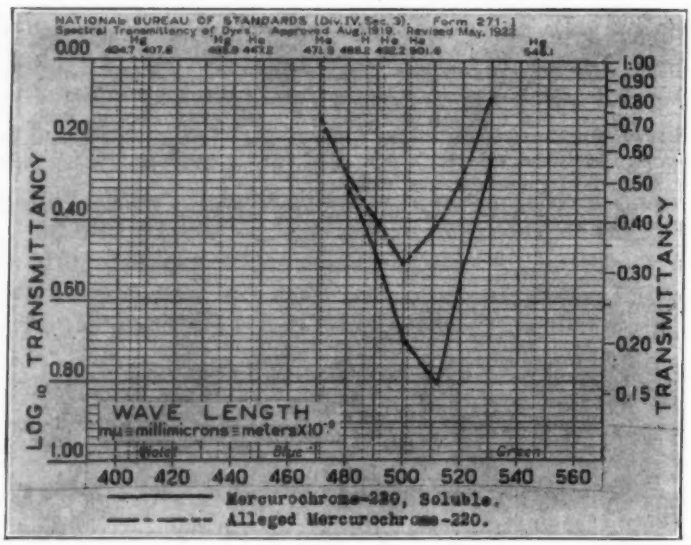

Fig. 1.

For the procurement of this drug the following specifications have been determined upon:

(a) Mercurochrome-220, soluble, shall be the disodium salt of dibromohydroxymercury-fluorescein.

(b) It shall be readily and completely soluble in water. Insoluble in alcohol (absolute).

(c) In general appearance the substance shall consist of greenish-appearing pseudocrystals and scales.

(d) On adding a few crystals to concentrated sulphuric acid a yellow solution is formed which on dilution with water gives a light orange precipitate. No change on adding to a 10 per cent aqueous solution of sodium hydroxide.

(e) Examined spectrophotometrically in a concentration of 10 parts of the dye to the million of phosphate buffer, P_H 7.0, a well-defined maximum

[2]Formanek, Untersuchung und Nachweis Organischer Farbstoffe auf Spektroskopischen Wege, Berlin, 1908.

shall occur at 512 mμ. The ratio between the transmissive indices at 500 mμ and 512 mμ shall be as 30 is to 34. For quantitative purposes such a solution shall show a transmissive index of 0.804 ± 0.050 at 512 mμ and of 0.700 ± 0.050 at 500 mμ when examined in a layer 1 cm. in thickness.

(f) When examined by methods common to the analysis of organic compounds, mercurochrome-220, soluble, shall show not less than 24 per cent or more than 26 per cent by weight of mercury on the dry basis.

Methods of inspection, tests, et cetera:

Mercurochrome-220, soluble, shall be tested according to the requirements of b, c, d, e, and f above. Values of the transmissive indices of a solution of mercurochrome-220, soluble, in phosphate buffer, P_H 7.0, 1.0 cg. of the dye to the liter of solvent when examined in a cell of 1 cm. thickness follows:

480mμ	0.322
490mμ	0.481
500mμ	0.700
510mμ	0.793
512mμ	0.804
520mμ	0.535
530mμ	0.240

These values should not vary more than a ±0.050 and there shall be no secondary maxima in the absorption curve. The maintenance of the ratio between the transmissive indices at 500 mμ and at 512 mμ as 30 is to 34 is important, as any increase in the value of the first member of this ratio indicates the presence of the color base, dibromfluorescein, which is undesirable.

The accompanying figure shows the curve of mercurochrome-220, soluble, plotted on the above values, as well as the curve of an alleged mercurochrome-220, soluble, which was on the market for a short time. It will be noted that this latter compound utterly failed to fill any of the above requirements and that spectrophotometrically it is in no sense the same substance. It showed 31 per cent mercury on analysis; and was only partially soluble in water.

Of the 13 samples submitted by White all met the above requirements easily and all had had clinical application.

POLYCHROME STAINS*

I. A Substitute for Giemsa's Stain

By R. W. French, Washington, D. C.

AFTER considerable experimenting with, and study of numerous samples of azure I, and the resultant azure II and azure II-eosin on the market, the conclusion has been reached that the production of a consistent Giemsa stain by the older methods is out of the question and illogical. The azures never agree in composition, and it is not believed that any practical method for the alkaline oxidation of methylene blue will result in a uniform product. Oxidation plays too important a part in the reaction.

From the methods of alkaline reduction, attention was turned to the acid reduction methods which have been studied quite thoroughly by MacNeal.[1] Here definite products of lower methylation are obtainable from methylene blue and the processes are thoroughly reproducible. It also is practical to consistently manufacture methylene violet (Bernthsen) of a practical degree of purity.

Originally MacNeal's study showed that certain of the lower homologues of methylene blue with methylene violet are an essential in a satisfactory polychrome blood stain. This has been confirmed by the spectrophotometer, and it is noted that a stain in order to produce satisfactory results must have a certain definite proportion of the several blue elements. This is supplied by MacNeal in his tetrachrome blood stain by definite amounts of methylene blue, methylene azure A (MacNeal) and methylene violet.

Consideration of a Giemsa stain and the many difficulties experienced in its preparation in the customary manner from azure II and azure II-eosin in view of the marked variation of these components has lead to a solution of the problem in a manner similar to that used by MacNeal[2] in his blood stain. A substitute for Giemsa of the following composition is offered. This advantage is claimed: it has certain definite components and can be consistently prepared.

Eosin	1.250	gm.
Methylene Blue	1.250	gm.
Methylene Azure A (MacNeal)	0.750	gm.
Methylene Violet (Bernthsen)	0.250	gm.
Methyl alcohol, absolute	375.0	ml.
Glycerin (anhydrous)	125.0	ml.

The proportion of the components is the same as that used by MacNeal in the tetrachrome stain though the concentration is much greater to allow of

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the customary Giemsa technic. These proportions give the correct blue elements for a complete differential staining of the basic components.

While the stain can be prepared by the above formula, some difficulty may be experienced unless a special quality of glycerin is used—the usual samples of glycerin are not sufficiently dry. It is found that this quality in the glycerin is an important consideration. A freshly diluted stain will work better than one which has stood for some little time as the methylene violet precipitates out rapidly in aqueous solution, though it is an essential to a satisfactory stain.

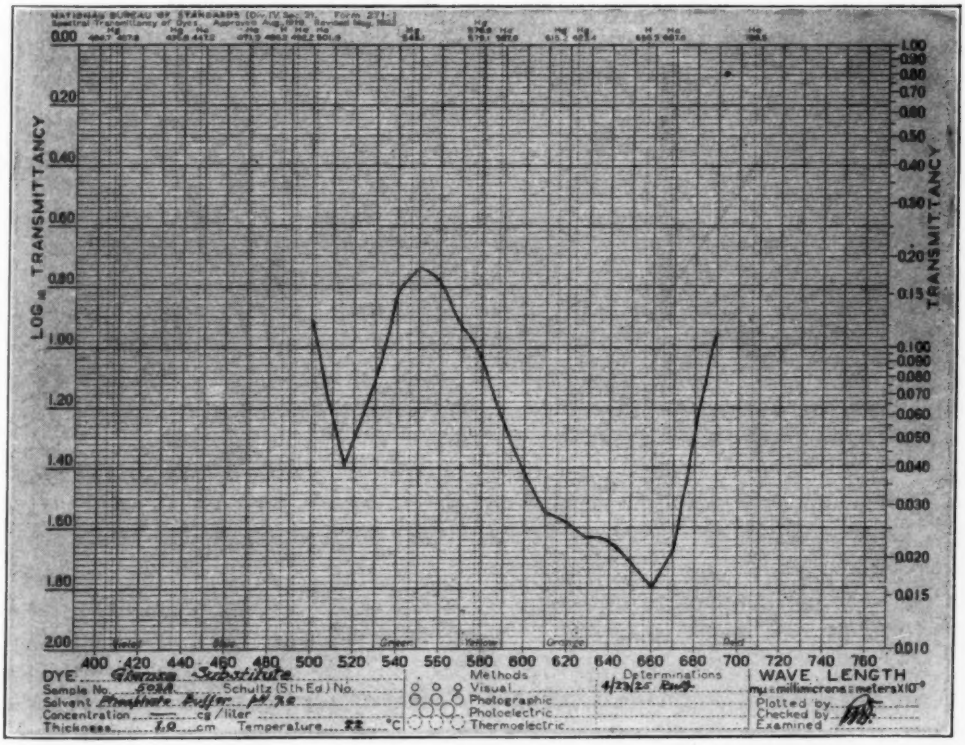

Fig. 1.

In use, a technic similar to that customarily employed with the original Giemsa is used; though controlled hydrogen-ion concentration is found to be advantageous for consistent results. In this latter method the above stain is used in the ratio of 3 ml. of stain solution to 50 ml. of a 50 per cent aqueous Sorensen phosphate buffer, P_H 7.5, and the slide is differentiated in a 50 per cent aqueous phosphate buffer of P_H 6.5.

Spectrophotometrically, as shown in the accompanying figure, this stain shows four distinct maxima of absorption: one at 516 mμ corresponding to eosin, one at 610 mμ due to the methylene violet, one at 630 mμ due to methylene azure and the greatest absorption at 660 mμ due to the methylene blue. Thus, the presence of the different elements is distinctly shown by this method of

analysis, and the relative amount of each may be ascertained. The study of different lots of stain compounded after the same formula shows a close agreement in the absorption curves and that the stain has a definite composition.

In another paper a discussion of the variations of Wright's stains, azures and Giemsa stains, on the market will be undertaken as revealed by the spectrophotometer.

REFERENCES

[1]MacNeal, Ward J.: Jour. Infec. Dis., 1906, iii, No. 3, 291-393.
[2]MacNeal, Ward J.: Jour. Am. Med. Assn., 1922, lxxviii, 1122-23.

NOTES ON BASAL METABOLISM*

VIII. Tables of Values of the Du Bois Surface Area Formula†

By William H. Stoner, A.M., M.D., Philadelphia, Pa.

DU BOIS and Du Bois,[1] presenting their formula for human surface area, constructed a graph of values of the formula in order to obviate the necessity of the logarithmic calculation in routine determinations. This "height-weight" chart covered heights from 100 to 200 centimeters and weights from 20 to 110 kilograms, and has been copied[2-8] more or less accurately in varying sizes and covering varying ranges of height and weight. Boothby and Sandiford[9] constructed nomograms, which have been reproduced,[10, 11] for calculating the values of surface area according to the Du Bois formula.

Considerable difficulty is experienced in reading these graphs and nomograms accurately to three significant figures even though they be constructed to large scale. Sanborn[2] has tabulated to three significant figures the values of the surface area formula for heights 140 to 200 cm. by 5 cm. intervals, and for weights 30 to 150 kg. by unit kg. intervals.

Many cases encountered in these hospitals fall without these height and weight limits and must be calculated logarithmically from the formula. Moreover, the necessary interpolation of Sanborn's values is inconvenient. For these reasons the values of the Du Bois formula are here tabulated over a greater height and weight range and by unit cm. and kg. intervals. The values are recorded to four significant figures, although sufficient accuracy for clinical determinations is given by three figures, since an error of a unit in the third place introduces an error of less than 1 per cent in the basal metabolic rate.

The result of a basal metabolic rate determination, although expressed usually in one or in two digits, is in fact a three digit value. For example, a basal metabolic rate of +5 means that the rate of heat production of the subject is 105 per cent of the normal or expected rate. The 5, therefore, is in reality a third digit which, to be correct, must be derived from factors correct to four significant figures. For this reason the temperature, the pressure, the volume of oxygen consumed, the calorific value of one liter of oxygen, the normal or standard of comparison, as well as the surface area,—

*From the Biochemistry Laboratories and the Departments of Metabolic Diseases of the Philadelphia General Hospital and of the Graduate School of Medicine of the University of Pennsylvania.

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†Preceding Notes of this series appeared in the Boston Medical and Surgical Journal as follows:

I. Modified Clinical Method of Determination, 1923, clxxxix, 193.
II. A Simplified Data Card for Clinical Determination, 1923, clxxxix, 195.
III. Errors of Clinical Determination, 1923, clxxxix, 232.
IV. Selection of Normal Standards, 1923, clxxxix, 236.
V. Tables of Values of Dreyer's Formulas, 1923, clxxxix, 239.
VI. Complementary Tables of Values of Dreyer's Formulas, 1924, cxci, 1026.
VII. Actual versus Theoretic Weight in Dreyer's Formulas, 1924, cxci, 1030.

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TABLE I

SURFACE AREA IN SQUARE METERS (DU BOIS FORMULA). 20 KG. TO 34 KG., 110 CM. TO 174 CM.

	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
110	0.775	0.791	0.807	0.823	0.838	0.852	0.867	0.881	0.894	0.908	0.921	0.934	0.947	0.959	0.971
111	0.780	0.796	0.812	0.828	0.843	0.858	0.872	0.886	0.900	0.914	0.927	0.940	0.953	0.965	0.978
112	0.785	0.802	0.818	0.833	0.848	0.863	0.878	0.892	0.906	0.920	0.933	0.946	0.959	0.972	0.984
113	0.790	0.807	0.823	0.839	0.854	0.869	0.884	0.898	0.912	0.926	0.939	0.952	0.965	0.978	0.991
114	0.795	0.812	0.828	0.844	0.860	0.875	0.889	0.904	0.918	0.932	0.945	0.959	0.972	0.984	0.997
115	0.800	0.817	0.833	0.849	0.865	0.880	0.895	0.909	0.923	0.937	0.951	0.965	0.978	0.991	1.003
116	0.806	0.823	0.839	0.855	0.871	0.886	0.901	0.915	0.929	0.943	0.957	0.971	0.984	0.997	1.010
117	0.811	0.828	0.844	0.860	0.876	0.891	0.906	0.921	0.935	0.949	0.963	0.977	0.990	1.003	1.016
118	0.816	0.833	0.849	0.865	0.881	0.897	0.912	0.927	0.941	0.955	0.969	0.983	0.996	1.009	1.022
119	0.821	0.838	0.854	0.871	0.887	0.902	0.917	0.932	0.947	0.961	0.975	0.989	1.002	1.015	1.028
120	0.826	0.843	0.860	0.876	0.892	0.908	0.923	0.938	0.952	0.967	0.981	0.995	1.008	1.022	1.035
121	0.831	0.848	0.865	0.881	0.898	0.913	0.928	0.943	0.958	0.973	0.987	1.001	1.014	1.028	1.041
122	0.836	0.853	0.870	0.887	0.903	0.919	0.934	0.949	0.964	0.978	0.993	1.007	1.020	1.034	1.047
123	0.841	0.858	0.875	0.892	0.908	0.924	0.939	0.955	0.970	0.984	0.999	1.013	1.027	1.040	1.053
124	0.845	0.863	0.880	0.897	0.914	0.930	0.945	0.960	0.975	0.990	1.004	1.019	1.033	1.046	1.059
125	0.850	0.868	0.886	0.903	0.919	0.935	0.951	0.966	0.981	0.996	1.010	1.025	1.039	1.052	1.066
126	0.855	0.873	0.891	0.908	0.924	0.940	0.956	0.972	0.987	1.002	1.016	1.031	1.045	1.059	1.072
127	0.860	0.878	0.896	0.913	0.930	0.946	0.962	0.977	0.992	1.007	1.022	1.037	1.051	1.065	1.078
128	0.865	0.883	0.901	0.918	0.935	0.951	0.967	0.983	0.998	1.013	1.028	1.043	1.057	1.071	1.084
129	0.870	0.888	0.906	0.923	0.940	0.957	0.973	0.989	1.004	1.019	1.034	1.048	1.063	1.077	1.090
130	0.875	0.893	0.911	0.928	0.945	0.962	0.978	0.994	1.009	1.024	1.039	1.054	1.069	1.083	1.096
131	0.880	0.898	0.916	0.934	0.951	0.968	0.984	0.999	1.015	1.030	1.045	1.060	1.075	1.089	1.103
132	0.885	0.903	0.921	0.939	0.956	0.973	0.989	1.005	1.020	1.036	1.051	1.066	1.080	1.095	1.109
133	0.889	0.908	0.926	0.944	0.961	0.978	0.994	1.010	1.026	1.042	1.057	1.072	1.086	1.101	1.115
134	0.894	0.913	0.931	0.949	0.966	0.983	0.999	1.016	1.032	1.047	1.062	1.077	1.092	1.107	1.121
135	0.899	0.918	0.936	0.954	0.972	0.989	1.005	1.021	1.037	1.053	1.068	1.083	1.098	1.113	1.127
136	0.904	0.923	0.941	0.959	0.977	0.994	1.010	1.027	1.043	1.059	1.074	1.089	1.104	1.119	1.133
137	0.909	0.928	0.946	0.964	0.982	0.999	1.016	1.032	1.048	1.064	1.080	1.095	1.110	1.125	1.139
138	0.914	0.933	0.951	0.969	0.987	1.004	1.021	1.037	1.054	1.070	1.085	1.101	1.116	1.131	1.145
139	0.919	0.938	0.956	0.975	0.993	1.010	1.027	1.043	1.060	1.076	1.091	1.107	1.122	1.136	1.151

140	0.923	0.942	0.961	0.980	0.998	1.015	1.032	1.049	1.065	1.081	1.097	1.112	1.127	1.142	1.157
141	0.928	0.947	0.966	0.985	1.003	1.020	1.037	1.054	1.071	1.087	1.103	1.118	1.133	1.148	1.163
142	0.933	0.952	0.971	0.990	1.008	1.026	1.043	1.060	1.076	1.092	1.108	1.124	1.139	1.154	1.169
143	0.937	0.957	0.976	0.995	1.013	1.031	1.048	1.065	1.081	1.098	1.114	1.130	1.145	1.160	1.175
144	0.942	0.962	0.981	1.000	1.018	1.036	1.053	1.070	1.087	1.103	1.119	1.135	1.151	1.166	1.181
145	0.947	0.967	0.986	1.005	1.023	1.041	1.059	1.076	1.092	1.109	1.125	1.141	1.157	1.172	1.187
146	0.951	0.971	0.991	1.010	1.029	1.047	1.064	1.081	1.098	1.115	1.131	1.147	1.163	1.178	1.193
147	0.956	0.976	0.996	1.015	1.034	1.052	1.069	1.086	1.103	1.120	1.136	1.152	1.168	1.184	1.199
148	0.961	0.981	1.001	1.020	1.039	1.057	1.074	1.092	1.109	1.126	1.142	1.158	1.174	1.190	1.205
149	0.966	0.986	1.006	1.025	1.044	1.062	1.080	1.097	1.114	1.131	1.148	1.164	1.179	1.195	1.210
150	0.971	0.991	1.011	1.030	1.049	1.067	1.085	1.102	1.119	1.136	1.153	1.169	1.185	1.201	1.216
151	0.975	0.996	1.016	1.035	1.054	1.073	1.090	1.108	1.125	1.142	1.159	1.175	1.191	1.207	1.222
152	0.980	1.001	1.021	1.040	1.059	1.078	1.096	1.114	1.131	1.148	1.165	1.181	1.197	1.213	1.228
153	0.985	1.005	1.025	1.045	1.064	1.083	1.101	1.119	1.136	1.153	1.170	1.187	1.203	1.218	1.234
154	0.989	1.010	1.030	1.050	1.069	1.088	1.106	1.124	1.141	1.158	1.175	1.192	1.208	1.224	1.240
155	0.994	1.014	1.035	1.055	1.074	1.093	1.111	1.129	1.147	1.164	1.181	1.198	1.214	1.230	1.246
156	0.999	1.019	1.040	1.060	1.079	1.098	1.116	1.134	1.152	1.169	1.186	1.203	1.220	1.236	1.251
157	1.003	1.024	1.045	1.065	1.084	1.103	1.121	1.139	1.157	1.175	1.192	1.209	1.225	1.241	1.257
158	1.008	1.029	1.050	1.070	1.089	1.108	1.127	1.145	1.163	1.181	1.198	1.215	1.231	1.247	1.263
159	1.013	1.034	1.054	1.074	1.094	1.113	1.132	1.150	1.168	1.186	1.203	1.220	1.237	1.253	1.269
160	1.017	1.038	1.059	1.079	1.099	1.118	1.137	1.156	1.174	1.192	1.209	1.226	1.243	1.259	1.275
161	1.022	1.043	1.064	1.084	1.104	1.123	1.142	1.161	1.179	1.197	1.214	1.231	1.248	1.264	1.280
162	1.026	1.048	1.069	1.089	1.109	1.128	1.147	1.166	1.184	1.202	1.220	1.237	1.253	1.270	1.286
163	1.031	1.053	1.074	1.094	1.114	1.133	1.152	1.171	1.190	1.208	1.225	1.242	1.259	1.276	1.292
164	1.036	1.057	1.078	1.099	1.119	1.138	1.157	1.176	1.195	1.213	1.230	1.247	1.264	1.281	1.297
165	1.040	1.062	1.083	1.104	1.124	1.144	1.163	1.182	1.200	1.218	1.236	1.253	1.270	1.287	1.303
166	1.045	1.066	1.088	1.109	1.129	1.149	1.168	1.187	1.205	1.223	1.241	1.259	1.276	1.293	1.309
167	1.049	1.071	1.092	1.113	1.134	1.154	1.173	1.192	1.210	1.229	1.247	1.264	1.281	1.298	1.315
168	1.054	1.076	1.097	1.118	1.139	1.159	1.178	1.197	1.216	1.234	1.252	1.270	1.287	1.304	1.320
169	1.058	1.080	1.102	1.123	1.143	1.163	1.183	1.202	1.221	1.239	1.257	1.275	1.292	1.309	1.326
170	1.063	1.085	1.107	1.128	1.148	1.168	1.188	1.207	1.226	1.245	1.263	1.281	1.298	1.315	1.332
171	1.067	1.089	1.111	1.132	1.153	1.173	1.193	1.212	1.231	1.250	1.268	1.286	1.303	1.320	1.337
172	1.072	1.094	1.116	1.137	1.158	1.178	1.198	1.218	1.237	1.255	1.273	1.291	1.309	1.326	1.343
173	1.076	1.098	1.120	1.141	1.162	1.183	1.203	1.223	1.242	1.260	1.278	1.296	1.314	1.331	1.348
174	1.080	1.103	1.125	1.146	1.167	1.188	1.208	1.228	1.247	1.265	1.284	1.302	1.320	1.337	1.354

TABLE II

SURFACE AREA IN SQUARE METERS (DU BOIS FORMULA). 35 KG. TO 49 KG., 120 CM. TO 184 CM.

	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
120	1.047	1.060	1.073	1.085	1.097	1.109	1.120	1.132	1.143	1.155	1.166	1.176	1.187	1.198	1.208
121	1.053	1.066	1.079	1.091	1.103	1.115	1.127	1.139	1.150	1.161	1.172	1.183	1.194	1.205	1.216
122	1.060	1.073	1.085	1.098	1.110	1.122	1.134	1.146	1.157	1.168	1.180	1.191	1.201	1.212	1.223
123	1.066	1.079	1.092	1.104	1.117	1.129	1.141	1.152	1.164	1.175	1.187	1.198	1.209	1.220	1.230
124	1.072	1.085	1.098	1.111	1.123	1.135	1.147	1.159	1.171	1.182	1.194	1.205	1.216	1.227	1.237
125	1.079	1.092	1.105	1.117	1.130	1.142	1.154	1.166	1.178	1.189	1.201	1.212	1.223	1.234	1.245
126	1.085	1.098	1.111	1.124	1.137	1.149	1.161	1.173	1.184	1.196	1.208	1.219	1.230	1.241	1.252
127	1.091	1.104	1.117	1.130	1.143	1.156	1.168	1.180	1.191	1.203	1.215	1.226	1.237	1.248	1.259
128	1.097	1.111	1.124	1.137	1.150	1.162	1.174	1.186	1.198	1.210	1.221	1.233	1.244	1.255	1.266
129	1.104	1.117	1.130	1.143	1.156	1.168	1.181	1.193	1.205	1.217	1.228	1.240	1.251	1.262	1.274
130	1.110	1.123	1.137	1.150	1.163	1.175	1.187	1.199	1.211	1.223	1.235	1.247	1.258	1.269	1.281
131	1.116	1.130	1.143	1.156	1.169	1.182	1.194	1.206	1.218	1.230.	1.242	1.254	1.265	1.277	1.288
132	1.122	1.136	1.149	1.162	1.175	1.188	1.200	1.213	1.225	1.237	1.249	1.261	1.272	1.284	1.295
133	1.128	1.142	1.156	1.169	1.182	1.195	1.207	1.220	1.232	1.244	1.256	1.268	1.279	1.291	1.302
134	1.134	1.148	1.162	1.175	1.188	1.201	1.213	1.226	1.238	1.250	1.262	1.274	1.286	1.298	1.309
135	1.141	1.155	1.168	1.181	1.194	1.207	1.220	1.233	1.245	1.257	1.269	1.281	1.293	1.305	1.316
136	1.147	1.161	1.174	1.188	1.201	1.214	1.227	1.239	1.252	1.264	1.276	1.288	1.300	1.312	1.323
137	1.153	1.167	1.181	1.194	1.207	1.220	1.233	1.246	1.258	1.271	1.283	1.295	1.307	1.319	1.330
138	1.159	1.173	1.187	1.200	1.214	1.227	1.240	1.253	1.265	1.278	1.290	1.302	1.314	1.326	1.337
139	1.165	1.179	1.193	1.207	1.220	1.233	1.246	1.260	1.272	1.284	1.297	1.309	1.321	1.333	1.344
140	1.171	1.185	1.199	1.213	1.227	1.240	1.253	1.266	1.278	1.291	1.304	1.316	1.327	1.339	1.351
141	1.177	1.192	1.206	1.219	1.233	1.246	1.259	1.272	1.285	1.298	1.310	1.323	1.334	1.346	1.358
142	1.183	1.198	1.212	1.225	1.239	1.253	1.266	1.279	1.292	1.304	1.317	1.329	1.341	1.353	1.365
143	1.189	1.204	1.218	1.232	1.246	1.259	1.272	1.285	1.298	1.311	1.323	1.336	1.348	1.360	1.372
144	1.195	1.210	1.224	1.238	1.252	1.265	1.278	1.292	1.305	1.318	1.330	1.343	1.355	1.367	1.379
145	1.201	1.216	1.230	1.244	1.258	1.272	1.285	1.298	1.311	1.324	1.337	1.350	1.362	1.374	1.386
146	1.207	1.222	1.237	1.251	1.265	1.278	1.292	1.305	1.318	1.331	1.344	1.356	1.369	1.381	1.393
147	1.213	1.228	1.243	1.257	1.271	1.284	1.298	1.311	1.324	1.338	1.350	1.363	1.375	1.388	1.400
148	1.219	1.234	1.249	1.263	1.277	1.291	1.304	1.318	1.331	1.344	1.357	1.370	1.382	1.395	1.407
149	1.225	1.240	1.255	1.269	1.283	1.297	1.311	1.324	1.338	1.351	1.364	1.377	1.389	1.402	1.414

150	1.231	1.246	1.261	1.275	1.289	1.303	1.317	1.331	1.344	1.357	1.370	1.383	1.396	1.408	1.421
151	1.237	1.252	1.267	1.281	1.296	1.310	1.323	1.337	1.351	1.364	1.377	1.390	1.402	1.415	1.428
152	1.243	1.258	1.273	1.287	1.302	1.316	1.329	1.343	1.357	1.370	1.383	1.396	1.409	1.422	1.434
153	1.249	1.264	1.279	1.294	1.308	1.322	1.336	1.350	1.363	1.377	1.390	1.403	1.416	1.429	1.441
154	1.255	1.270	1.285	1.300	1.314	1.328	1.342	1.356	1.370	1.383	1.396	1.409	1.422	1.435	1.448
155	1.261	1.276	1.291	1.306	1.321	1.335	1.349	1.363	1.376	1.390	1.403	1.416	1.429	1.442	1.455
156	1.267	1.282	1.297	1.312	1.327	1.341	1.355	1.369	1.383	1.396	1.410	1.423	1.436	1.449	1.462
157	1.273	1.288	1.303	1.318	1.333	1.347	1.361	1.375	1.389	1.403	1.416	1.429	1.442	1.455	1.468
158	1.279	1.294	1.309	1.324	1.339	1.354	1.368	1.382	1.396	1.410	1.423	1.436	1.449	1.462	1.475
159	1.285	1.300	1.315	1.330	1.345	1.360	1.374	1.388	1.402	1.416	1.429	1.443	1.456	1.469	1.482
160	1.291	1.306	1.321	1.336	1.351	1.366	1.380	1.395	1.409	1.423	1.436	1.450	1.463	1.476	1.489
161	1.296	1.312	1.327	1.342	1.357	1.372	1.386	1.401	1.415	1.429	1.442	1.456	1.469	1.482	1.495
162	1.302	1.318	1.333	1.348	1.363	1.378	1.393	1.407	1.421	1.435	1.449	1.463	1.476	1.489	1.502
163	1.308	1.324	1.339	1.354	1.369	1.384	1.399	1.414	1.428	1.442	1.455	1.469	1.483	1.496	1.509
164	1.313	1.329	1.345	1.360	1.375	1.390	1.405	1.420	1.434	1.448	1.462	1.475	1.489	1.502	1.516
165	1.319	1.335	1.351	1.366	1.382	1.397	1.411	1.426	1.440	1.454	1.468	1.482	1.496	1.509	1.522
166	1.325	1.341	1.357	1.372	1.388	1.403	1.417	1.432	1.446	1.461	1.475	1.489	1.502	1.516	1.529
167	1.331	1.347	1.363	1.378	1.394	1.409	1.423	1.438	1.452	1.467	1.481	1.495	1.508	1.522	1.536
168	1.337	1.353	1.369	1.384	1.400	1.415	1.430	1.444	1.459	1.473	1.487	1.501	1.515	1.529	1.542
169	1.342	1.359	1.375	1.390	1.406	1.421	1.436	1.451	1.465	1.480	1.494	1.508	1.522	1.535	1.548
170	1.348	1.364	1.380	1.396	1.412	1.427	1.442	1.457	1.471	1.486	1.500	1.514	1.528	1.542	1.555
171	1.354	1.370	1.386	1.402	1.418	1.433	1.448	1.463	1.478	1.492	1.507	1.521	1.535	1.548	1.562
172	1.360	1.376	1.392	1.408	1.424	1.439	1.454	1.469	1.484	1.499	1.513	1.527	1.541	1.555	1.569
173	1.365	1.382	1.398	1.414	1.430	1.445	1.460	1.476	1.490	1.505	1.519	1.533	1.547	1.561	1.575
174	1.371	1.388	1.404	1.420	1.436	1.451	1.467	1.482	1.497	1.511	1.526	1.540	1.554	1.568	1.582
175	1.377	1.393	1.410	1.426	1.442	1.457	1.473	1.488	1.503	1.518	1.532	1.547	1.561	1.575	1.589
176	1.383	1.399	1.416	1.432	1.448	1.464	1.479	1.494	1.509	1.524	1.539	1.553	1.567	1.581	1.595
177	1.388	1.405	1.422	1.438	1.454	1.470	1.485	1.500	1.515	1.530	1.545	1.560	1.574	1.588	1.602
178	1.394	1.411	1.428	1.444	1.460	1.476	1.491	1.507	1.522	1.537	1.552	1.566	1.581	1.595	1.609
179	1.400	1.417	1.434	1.450	1.466	1.482	1.497	1.513	1.528	1.543	1.558	1.573	1.587	1.601	1.615
180	1.405	1.422	1.439	1.455	1.471	1.487	1.503	1.518	1.534	1.549	1.564	1.579	1.593	1.607	1.621
181	1.411	1.428	1.445	1.461	1.477	1.493	1.509	1.525	1.540	1.555	1.570	1.585	1.599	1.614	1.628
182	1.417	1.434	1.451	1.467	1.483	1.499	1.515	1.531	1.546	1.561	1.576	1.591	1.606	1.620	1.635
183	1.422	1.439	1.456	1.473	1.489	1.505	1.521	1.537	1.552	1.567	1.582	1.597	1.612	1.626	1.641
184	1.428	1.445	1.462	1.479	1.495	1.511	1.527	1.543	1.558	1.574	1.589	1.604	1.618	1.633	1.647

TABLE III

SURFACE AREA IN SQUARE METERS (DU BOIS FORMULA). 50 KG. TO 64 KG., 135 CM. TO 199 CM.

	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64
135	1.327	1.338	1.349	1.361	1.372	1.383	1.393	1.404	1.414	1.424	1.434	1.445	1.455	1.465	1.475
136	1.335	1.346	1.357	1.368	1.379	1.390	1.401	1.411	1.421	1.432	1.442	1.452	1.463	1.472	1.482
137	1.342	1.353	1.364	1.375	1.386	1.397	1.408	1.419	1.429	1.439	1.450	1.460	1.470	1.480	1.490
138	1.349	1.360	1.371	1.383	1.394	1.405	1.415	1.426	1.436	1.447	1.457	1.468	1.478	1.488	1.498
139	1.356	1.367	1.378	1.390	1.401	1.412	1.423	1.434	1.444	1.455	1.465	1.476	1.486	1.496	1.506
140	1.363	1.374	1.385	1.397	1.408	1.419	1.430	1.441	1.451	1.462	1.473	1.483	1.493	1.503	1.514
141	1.370	1.381	1.393	1.404	1.416	1.427	1.438	1.449	1.459	1.470	1.480	1.491	1.501	1.511	1.522
142	1.377	1.388	1.400	1.412	1.423	1.434	1.445	1.456	1.467	1.477	1.488	1.499	1.509	1.519	1.529
143	1.384	1.395	1.407	1.419	1.430	1.441	1.452	1.463	1.474	1.485	1.496	1.506	1.517	1.527	1.537
144	1.391	1.402	1.414	1.426	1.437	1.449	1.460	1.471	1.482	1.492	1.503	1.514	1.524	1.535	1.545
145	1.398	1.410	1.421	1.433	1.445	1.456	1.467	1.478	1.489	1.500	1.511	1.522	1.532	1.542	1.553
146	1.405	1.417	1.428	1.440	1.452	1.464	1.475	1.486	1.497	1.508	1.518	1.529	1.540	1.550	1.561
147	1.412	1.424	1.435	1.447	1.459	1.471	1.482	1.493	1.504	1.515	1.526	1.537	1.547	1.558	1.568
148	1.419	1.431	1.443	1.455	1.467	1.478	1.489	1.500	1.511	1.523	1.534	1.545	1.555	1.566	1.576
149	1.426	1.438	1.450	1.462	1.474	1.485	1.497	1.508	1.519	1.530	1.541	1.552	1.563	1.573	1.584
150	1.433	1.445	1.457	1.469	1.481	1.492	1.504	1.515	1.526	1.537	1.548	1.559	1.570	1.581	1.591
151	1.440	1.452	1.464	1.476	1.488	1.500	1.511	1.523	1.534	1.545	1.556	1.567	1.578	1.589	1.599
152	1.447	1.459	1.471	1.483	1.495	1.507	1.518	1.530	1.541	1.552	1.563	1.574	1.585	1.596	1.607
153	1.454	1.466	1.478	1.490	1.502	1.514	1.525	1.537	1.548	1.560	1.571	1.582	1.593	1.604	1.614
154	1.461	1.473	1.485	1.497	1.509	1.521	1.532	1.544	1.555	1.567	1.578	1.589	1.600	1.611	1.622
155	1.468	1.480	1.492	1.504	1.516	1.528	1.540	1.552	1.563	1.574	1.586	1.597	1.608	1.619	1.630
156	1.474	1.486	1.498	1.511	1.523	1.535	1.547	1.559	1.570	1.582	1.593	1.604	1.615	1.626	1.637
157	1.481	1.493	1.505	1.518	1.530	1.542	1.554	1.566	1.577	1.589	1.600	1.612	1.623	1.634	1.645
158	1.488	1.500	1.512	1.525	1.538	1.550	1.562	1.573	1.585	1.596	1.608	1.620	1.631	1.642	1.653
159	1.495	1.507	1.519	1.532	1.545	1.557	1.569	1.581	1.592	1.604	1.615	1.627	1.638	1.649	1.660
160	1.502	1.514	1.526	1.539	1.552	1.564	1.576	1.588	1.599	1.611	1.623	1.635	1.646	1.657	1.668
161	1.508	1.521	1.533	1.546	1.559	1.571	1.583	1.595	1.606	1.618	1.630	1.641	1.653	1.664	1.675
162	1.515	1.528	1.540	1.553	1.566	1.578	1.590	1.602	1.614	1.626	1.637	1.649	1.660	1.671	1.683
163	1.522	1.535	1.547	1.560	1.573	1.585	1.597	1.609	1.621	1.633	1.645	1.657	1.668	1.679	1.690
164	1.529	1.541	1.554	1.567	1.580	1.592	1.604	1.616	1.628	1.640	1.652	1.664	1.675	1.686	1.697

165	1.535	1.548	1.561	1.574	1.587	1.599	1.611	1.624	1.635	1.647	1.659	1.671	1.683	1.694	1.705
166	1.542	1.555	1.568	1.581	1.594	1.606	1.618	1.631	1.642	1.654	1.666	1.678	1.690	1.701	1.713
167	1.549	1.561	1.574	1.587	1.600	1.613	1.625	1.638	1.649	1.661	1.673	1.685	1.697	1.708	1.720
168	1.555	1.568	1.581	1.594	1.607	1.620	1.632	1.645	1.657	1.669	1.681	1.693	1.705	1.716	1.727
169	1.562	1.575	1.588	1.601	1.614	1.627	1.639	1.652	1.664	1.676	1.688	1.700	1.712	1.723	1.735
170	1.569	1.582	1.595	1.608	1.621	1.634	1.646	1.659	1.671	1.683	1.695	1.707	1.719	1.731	1.742
171	1.576	1.589	1.602	1.615	1.628	1.641	1.653	1.666	1.678	1.690	1.703	1.715	1.727	1.738	1.750
172	1.583	1.596	1.609	1.622	1.635	1.648	1.660	1.673	1.686	1.698	1.710	1.722	1.734	1.745	1.757
173	1.589	1.602	1.615	1.628	1.642	1.655	1.667	1.680	1.693	1.705	1.717	1.729	1.741	1.753	1.765
174	1.596	1.609	1.622	1.635	1.649	1.662	1.675	1.687	1.699	1.712	1.724	1.737	1.749	1.760	1.772
175	1.603	1.616	1.629	1.642	1.656	1.669	1.682	1.694	1.706	1.719	1.731	1.744	1.756	1.768	1.780
176	1.609	1.622	1.635	1.649	1.663	1.676	1.689	1.701	1.713	1.726	1.739	1.751	1.763	1.775	1.787
177	1.616	1.629	1.642	1.656	1.670	1.683	1.696	1.708	1.720	1.733	1.746	1.758	1.771	1.782	1.794
178	1.623	1.636	1.649	1.663	1.677	1.690	1.703	1.715	1.728	1.740	1.753	1.766	1.778	1.790	1.802
179	1.629	1.642	1.656	1.670	1.683	1.697	1.710	1.722	1.735	1.747	1.760	1.773	1.785	1.797	1.809
180	1.635	1.649	1.662	1.676	1.690	1.703	1.716	1.729	1.742	1.754	1.767	1.780	1.792	1.804	1.816
181	1.642	1.655	1.669	1.683	1.697	1.710	1.723	1.736	1.749	1.762	1.774	1.787	1.799	1.811	1.823
182	1.649	1.662	1.676	1.690	1.704	1.717	1.730	1.743	1.756	1.769	1.781	1.794	1.806	1.819	1.831
183	1.655	1.668	1.682	1.696	1.710	1.723	1.737	1.750	1.763	1.776	1.788	1.801	1.813	1.826	1.838
184	1.661	1.675	1.689	1.703	1.717	1.730	1.744	1.757	1.770	1.783	1.796	1.808	1.821	1.833	1.845
185	1.668	1.682	1.696	1.710	1.724	1.737	1.751	1.764	1.777	1.790	1.803	1.816	1.828	1.840	1.853
186	1.675	1.688	1.702	1.717	1.731	1.744	1.758	1.771	1.784	1.797	1.810	1.823	1.835	1.848	1.860
187	1.681	1.695	1.709	1.723	1.737	1.751	1.764	1.778	1.791	1.804	1.817	1.830	1.842	1.855	1.867
188	1.688	1.702	1.716	1.730	1.744	1.758	1.771	1.785	1.798	1.811	1.824	1.837	1.849	1.862	1.875
189	1.695	1.708	1.722	1.737	1.751	1.765	1.778	1.792	1.805	1.818	1.831	1.844	1.856	1.869	1.882
190	1.701	1.715	1.729	1.743	1.758	1.771	1.785	1.798	1.811	1.825	1.838	1.851	1.863	1.876	1.889
191	1.707	1.721	1.735	1.750	1.764	1.778	1.791	1.805	1.818	1.832	1.845	1.858	1.870	1.883	1.896
192	1.714	1.728	1.742	1.757	1.771	1.785	1.798	1.812	1.825	1.839	1.852	1.865	1.878	1.891	1.903
193	1.721	1.735	1.749	1.764	1.778	1.792	1.805	1.819	1.832	1.846	1.859	1.872	1.885	1.898	1.911
194	1.727	1.741	1.755	1.770	1.784	1.798	1.812	1.826	1.839	1.853	1.866	1.879	1.892	1.905	1.918
195	1.733	1.747	1.762	1.777	1.791	1.805	1.819	1.833	1.846	1.860	1.873	1.886	1.899	1.912	1.925
196	1.740	1.754	1.768	1.783	1.798	1.812	1.826	1.840	1.853	1.866	1.880	1.893	1.906	1.919	1.832
197	1.746	1.760	1.775	1.790	1.804	1.818	1.832	1.846	1.860	1.873	1.887	1.900	1.913	1.926	1.939
198	1.752	1.767	1.781	1.796	1.811	1.825	1.839	1.853	1.866	1.880	1.894	1.907	1.920	1.933	1.946
199	1.758	1.773	1.788	1.803	1.818	1.832	1.846	1.860	1.873	1.887	1.900	1.913	1.926	1.940	1.953

Table IV

Surface Area in Square Meters (Du Bois Formula). 65 kg. to 79 kg., 135 cm. to 199 cm.

	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79
135	1.484	1.494	1.503	1.513	1.522	1.532	1.541	1.550	1.559	1.568	1.577	1.586	1.595	1.604	1.613
136	1.492	1.502	1.511	1.521	1.530	1.540	1.549	1.558	1.567	1.577	1.586	1.595	1.604	1.612	1.621
137	1.500	1.510	1.519	1.529	1.539	1.548	1.557	1.567	1.576	1.585	1.594	1.603	1.612	1.621	1.630
138	1.508	1.518	1.528	1.537	1.547	1.556	1.566	1.575	1.584	1.593	1.603	1.611	1.620	1.629	1.638
139	1.516	1.526	1.536	1.545	1.555	1.565	1.574	1.583	1.593	1.602	1.611	1.620	1.629	1.638	1.647
140	1.524	1.534	1.544	1.553	1.563	1.573	1.582	1.591	1.601	1.610	1.619	1.628	1.637	1.646	1.655
141	1.532	1.542	1.552	1.561	1.571	1.581	1.590	1.600	1.609	1.618	1.628	1.637	1.646	1.655	1.664
142	1.540	1.550	1.560	1.569	1.579	1.589	1.598	1.608	1.617	1.627	1.636	1.645	1.654	1.663	1.673
143	1.547	1.557	1.567	1.577	1.587	1.597	1.607	1.616	1.625	1.635	1.644	1.653	1.663	1.672	1.681
144	1.555	1.565	1.575	1.585	1.595	1.605	1.615	1.624	1.634	1.643	1.653	1.662	1.671	1.680	1.690
145	1.563	1.573	1.583	1.593	1.603	1.613	1.623	1.633	1.642	1.652	1.661	1.670	1.680	1.689	1.698
146	1.571	1.581	1.591	1.601	1.611	1.621	1.631	1.641	1.650	1.660	1.670	1.679	1.688	1.697	1.707
147	1.579	1.589	1.599	1.609	1.619	1.629	1.639	1.649	1.658	1.668	1.678	1.687	1.697	1.706	1.715
148	1.587	1.597	1.607	1.617	1.627	1.637	1.647	1.657	1.667	1.676	1.686	1.696	1.705	1.714	1.724
149	1.594	1.605	1.615	1.625	1.635	1.645	1.655	1.665	1.675	1.685	1.694	1.704	1.714	1.723	1.732
150	1.602	1.613	1.623	1.633	1.643	1.653	1.663	1.673	1.683	1.693	1.703	1.712	1.722	1.731	1.741
151	1.610	1.620	1.631	1.641	1.651	1.661	1.671	1.681	1.691	1.701	1.711	1.720	1.730	1.739	1.749
152	1.617	1.628	1.638	1.649	1.659	1.669	1.679	1.689	1.699	1.709	1.719	1.728	1.738	1.747	1.757
153	1.625	1.636	1.646	1.657	1.667	1.677	1.687	1.697	1.707	1.717	1.727	1.737	1.747	1.756	1.766
154	1.633	1.643	1.654	1.664	1.675	1.685	1.695	1.705	1.715	1.725	1.735	1.745	1.755	1.764	1.774
155	1.641	1.651	1.662	1.672	1.683	1.693	1.703	1.713	1.723	1.733	1.743	1.753	1.763	1.773	1.782
156	1.648	1.659	1.670	1.680	1.690	1.701	1.711	1.721	1.731	1.741	1.751	1.761	1.771	1.781	1.791
157	1.656	1.666	1.677	1.688	1.698	1.709	1.719	1.729	1.739	1.749	1.760	1.770	1.780	1.789	1.799
158	1.664	1.675	1.685	1.696	1.706	1.717	1.727	1.737	1.747	1.758	1.768	1.778	1.788	1.798	1.808
159	1.671	1.682	1.693	1.703	1.714	1.725	1.735	1.745	1.755	1.766	1.776	1.786	1.796	1.806	1.816
160	1.679	1.690	1.701	1.711	1.722	1.733	1.743	1.753	1.763	1.774	1.784	1.794	1.804	1.814	1.824
161	1.686	1.697	1.708	1.719	1.729	1.740	1.751	1.761	1.771	1.782	1.792	1.802	1.812	1.822	1.832
162	1.694	1.705	1.716	1.727	1.737	1.748	1.759	1.769	1.780	1.790	1.800	1.810	1.820	1.830	1.840
163	1.702	1.713	1.724	1.735	1.745	1.756	1.767	1.777	1.788	1.798	1.808	1.818	1.829	1.839	18.49
164	1.709	1.720	1.731	1.742	1.753	1.764	1.774	1.785	1.795	1.806	1.816	1.826	1.837	1.847	1.857

165	1.717	1.728	1.739	1.750	1.761	1.772	1.782	1.793	1.803	1.814	1.824	1.834	1.845	1.855	1.865
166	1.724	1.735	1.747	1.758	1.769	1.780	1.790	1.801	1.811	1.822	1.832	1.842	1.853	1.863	1.873
167	1.731	1.743	1.754	1.765	1.776	1.787	1.798	1.808	1.819	1.829	1.840	1.850	1.861	1.871	1.881
168	1.739	1.750	1.762	1.773	1.784	1.795	1.806	1.816	1.827	1.837	1.848	1.858	1.869	1.879	1.889
169	1.747	1.758	1.769	1.780	1.791	1.802	1.813	1.824	1.835	1.845	1.856	1.866	1.877	1.887	1.897
170	1.754	1.765	1.777	1.788	1.799	1.810	1.821	1.832	1.843	1.853	1.864	1.874	1.885	1.895	1.905
171	1.762	1.773	1.785	1.796	1.807	1.818	1.829	1.840	1.851	1.861	1.872	1.882	1.893	1.903	1.914
172	1.769	1.781	1.792	1.803	1.814	1.826	1.837	1.848	1.859	1.869	1.880	1.890	1.901	1.912	1.922
173	1.777	1.788	1.799	1.810	1.821	1.833	1.844	1.855	1.866	1.877	1.888	1.898	1.909	1.920	1.930
174	1.784	1.796	1.807	1.818	1.829	1.841	1.852	1.863	1.874	1.885	1.896	1.906	1.917	1.928	1.938
175	1.791	1.803	1.815	1.826	1.837	1.849	1.860	1.871	1.882	1.893	1.904	1.914	1.925	1.936	1.946
176	1.799	1.811	1.822	1.834	1.845	1.857	1.868	1.879	1.890	1.901	1.912	1.922	1.933	1.944	1.954
177	1.806	1.818	1.830	1.841	1.853	1.864	1.876	1.887	1.898	1.909	1.920	1.930	1.941	1.952	1.962
178	1.814	1.826	1.838	1.849	1.861	1.872	1.884	1.895	1.906	1.917	1.928	1.938	1.949	1.960	1.971
179	1.821	1.833	1.845	1.857	1.868	1.880	1.891	1.902	1.913	1.924	1.935	1.946	1.957	1.968	1.979
180	1.828	1.840	1.852	1.864	1.875	1.887	1.898	1.909	1.920	1.932	1.943	1.954	1.965	1.975	1.986
181	1.836	1.848	1.860	1.871	1.883	1.895	1.906	1.917	1.928	1.940	1.951	1.962	1.973	1.983	1.994
182	1.843	1.855	1.867	1.879	1.891	1.902	1.914	1.925	1.936	1.948	1.959	1.970	1.981	1.991	2.002
183	1.850	1.862	1.874	1.886	1.898	1.909	1.921	1.932	1.944	1.955	1.966	1.977	1.988	1.999	2.010
184	1.858	1.870	1.882	1.894	1.905	1.917	1.929	1.940	1.952	1.963	1.974	1.985	1.996	2.007	2.018
185	1.865	1.877	1.889	1.901	1.913	1.925	1.936	1.948	1.959	1.971	1.982	1.993	2.004	2.015	2.026
186	1.872	1.885	1.897	1.909	1.921	1.932	1.944	1.956	1.967	1.978	1.990	2.001	2.012	2.023	2.034
187	1.879	1.892	1.904	1.916	1.928	1.940	1.951	1.963	1.974	1.986	1.997	2.008	2.020	2.031	2.042
188	1.887	1.899	1.912	1.924	1.936	1.948	1.959	1.971	1.982	1.994	2.005	2.016	2.028	2.039	2.050
189	1.895	1.907	1.919	1.931	1.943	1.955	1.967	1.979	1.990	2.002	2.013	2.024	2.036	2.047	2.058
190	1.902	1.914	1.926	1.938	1.950	1.962	1.974	1.986	1.998	2.009	2.021	2.032	2.044	2.055	2.066
191	1.909	1.921	1.933	1.945	1.957	1.970	1.982	1.994	2.005	2.017	2.028	2.039	2.051	2.062	2.074
192	1.916	1.928	1.941	1.953	1.965	1.977	1.989	2.001	2.013	2.024	2.036	2.047	2.059	2.070	2.082
193	1.924	1.936	1.948	1.961	1.973	1.985	1.997	2.009	2.021	2.032	2.044	2.055	2.067	2.078	2.090
194	1.931	1.943	1.956	1.968	1.980	1.992	2.004	2.016	2.028	2.040	2.052	2.063	2.075	2.086	2.097
195	1.938	1.950	1.963	1.975	1.987	2.000	2.012	2.024	2.036	2.047	2.059	2.071	2.083	2.094	2.105
196	1.945	1.957	1.970	1.982	1.995	2.007	2.019	2.031	2.043	2.055	2.067	2.078	2.090	2.101	2.113
197	1.952	1.965	1.977	1.990	2.002	2.015	2.027	2.039	2.051	2.063	2.074	2.086	2.097	2.109	2.121
198	1.959	1.972	1.985	1.997	2.010	2.022	2.034	2.046	2.058	2.070	2.082	2.094	2.105	2.117	2.129
199	1.966	1.979	1.992	2.004	2.017	2.030	2.042	2.054	2.066	2.078	2.090	2.101	2.113	2.125	2.137

TABLE V

SURFACE AREA IN SQUARE METERS (DU BOIS FORMULA). 80 KG. TO 94 KG., 135 CM. TO 199 CM.

	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94
135	1.621	1.629	1.638	1.647	1.655	1.664	1.672	1.680	1.688	1.696	1.704	1.712	1.720	1.728	1.736
136	1.630	1.638	1.647	1.655	1.664	1.673	1.681	1.689	1.697	1.705	1.713	1.721	1.729	1.737	1.745
137	1.638	1.646	1.655	1.664	1.673	1.682	1.690	1.698	1.706	1.714	1.722	1.731	1.739	1.747	1.755
138	1.647	1.655	1.664	1.673	1.682	1.690	1.699	1.707	1.715	1.723	1.731	1.740	1.748	1.756	1.764
139	1.656	1.664	1.673	1.682	1.690	1.699	1.708	1.716	1.724	1.733	1.741	1.749	1.757	1.765	1.773
140	1.664	1.672	1.681	1.690	1.699	1.708	1.716	1.725	1.733	1.742	1.750	1.758	1.766	1.774	1.782
141	1.673	1.681	1.690	1.699	1.708	1.717	1.725	1.734	1.742	1.751	1.759	1.767	1.775	1.784	1.792
142	1.682	1.690	1.699	1.708	1.717	1.726	1.734	1.743	1.751	1.760	1.768	1.776	1.784	1.793	1.801
143	1.690	1.699	1.708	1.717	1.725	1.735	1.743	1.751	1.760	1.769	1.777	1.785	1.793	1.802	1.810
144	1.699	1.707	1.716	1.725	1.734	1.743	1.752	1.760	1.769	1.778	1.786	1.794	1.803	1.811	1.819
145	1.707	1.716	1.725	1.734	1.743	1.752	1.761	1.769	1.778	1.787	1.795	1.803	1.812	1.820	1.828
146	1.716	1.725	1.734	1.743	1.752	1.761	1.770	1.778	1.787	1.796	1.804	1.813	1.821	1.829	1.837
147	1.724	1.733	1.742	1.751	1.760	1.770	1.778	1.787	1.796	1.805	1.813	1.822	1.830	1.838	1.846
148	1.733	1.742	1.751	1.760	1.769	1.779	1.787	1.796	1.805	1.814	1.822	1.831	1.839	1.848	1.856
149	1.741	1.750	1.760	1.769	1.778	1.787	1.796	1.805	1.813	1.822	1.831	1.840	1.848	1.857	1.865
150	1.750	1.759	1.768	1.777	1.786	1.796	1.805	1.814	1.822	1.831	1.840	1.848	1.857	1.866	1.874
151	1.758	1.767	1.777	1.786	1.795	1.805	1.814	1.823	1.831	1.840	1.848	1.857	1.866	1.875	1.883
152	1.766	1.775	1.785	1.794	1.803	1.813	1.822	1.831	1.840	1.849	1.857	1.866	1.875	1.883	1.892
153	1.775	1.784	1.794	1.803	1.812	1.822	1.831	1.840	1.848	1.857	1.866	1.875	1.884	1.892	1.901
154	1.783	1.792	1.802	1.811	1.821	1.830	1.839	1.848	1.857	1.866	1.875	1.884	1.893	1.901	1.910
155	1.792	1.801	1.811	1.820	1.829	1.839	1.848	1.857	1.866	1.875	1.884	1.893	1.902	1.910	1.919
156	1.800	1.809	1.819	1.829	1.838	1.848	1.857	1.866	1.875	1.884	1.893	1.902	1.910	1.919	1.928
157	1.808	1.818	1.828	1.837	1.846	1.856	1.865	1.874	1.883	1.892	1.901	1.910	1.919	1.928	1.937
158	1.817	1.827	1.837	1.846	1.855	1.865	1.874	1.883	1.892	1.901	1.910	1.919	1.928	1.937	1.946
159	1.825	1.835	1.845	1.854	1.863	1.873	1.882	1.891	1.901	1.910	1.919	1.928	1.937	1.946	1.955
160	1.834	1.844	1.854	1.863	1.872	1.882	1.891	1.900	1.910	1.919	1.928	1.937	1.946	1.955	1.964
161	1.842	1.852	1.862	1.871	1.880	1.890	1.899	1.909	1.918	1.927	1.936	1.945	1.954	1.963	1.972
162	1.850	1.860	1.870	1.879	1.889	1.899	1.908	1.917	1.927	1.936	1.945	1.954	1.963	1.972	1.981
163	1.859	1.868	1.878	1.888	1.898	1.908	1.917	1.926	1.936	1.945	1.954	1.963	1.972	1.981	1.990
164	1.867	1.876	1.886	1.896	1.906	1.916	1.925	1.934	1.944	1.953	1.962	1.972	1.981	1.990	1.999

165	1.875	1.885	1.895	1.905	1.914	1.924	1.934	1.943	1.953	1.962	1.971	1.981	1.990	1.999	2.008
166	1.883	1.893	1.903	1.913	1.923	1.933	1.942	1.952	1.961	1.971	1.980	1.989	1.998	2.008	2.017
167	1.891	1.901	1.911	1.921	1.931	1.941	1.950	1.960	1.969	1.979	1.988	1.998	2.007	2.016	2.025
168	1.899	1.909	1.920	1.930	1.939	1.949	1.959	1.968	1.978	1.988	1.997	2.006	2.016	2.025	2.034
169	1.908	1.918	1.928	1.938	1.948	1.958	1.967	1.977	1.987	1.996	2.005	2.015	2.024	2.034	2.043
170	1.916	1.926	1.936	1.946	1.956	1.966	1.976	1.985	1.995	2.005	2.014	2.023	2.033	2.042	2.051
171	1.924	1.934	1.944	1.954	1.964	1.974	1.984	1.994	2.004	2.014	2.023	2.032	2.042	2.051	2.060
172	1.932	1.942	1.953	1.963	1.973	1.983	1.993	2.003	2.012	2.022	2.031	2.041	2.051	2.060	2.069
173	1.940	1.950	1.961	1.971	1.981	1.991	2.001	2.011	2.020	2.030	2.039	2.049	2.059	2.068	2.077
174	1.948	1.958	1.969	1.979	1.989	2.000	2.010	2.019	2.029	2.039	2.048	2.058	2.068	2.077	2.086
175	1.957	1.967	1.977	1.987	1.998	2.008	2.018	2.028	2.038	2.048	2.057	2.067	2.076	2.086	2.095
176	1.965	1.975	1.986	1.996	2.006	2.017	2.026	2.036	2.046	2.056	2.065	2.075	2.085	2.095	2.104
177	1.973	1.983	1.994	2.004	2.014	2.025	2.035	2.045	2.054	2.064	2.074	2.084	2.094	2.103	2.113
178	1.981	1.991	2.002	2.012	2.023	2.034	2.044	2.054	2.063	2.073	2.083	2.093	2.103	2.112	2.122
179	1.989	1.999	2.010	2.020	2.031	2.042	2.052	2.062	2.072	2.082	2.091	2.101	2.111	2.121	2.130
180	1.997	2.007	2.018	2.028	2.039	2.050	2.060	2.070	2.080	2.090	2.099	2.109	2.119	2.129	2.138
181	2.005	2.015	2.026	2.037	2.047	2.058	2.068	2.078	2.088	2.098	2.108	2.118	2.128	2.137	2.147
182	2.013	2.023	2.034	2.045	2.055	2.066	2.076	2.086	2.097	2.107	2.116	2.126	2.136	2.146	2.156
183	2.021	2.031	2.042	2.053	2.063	2.074	2.084	2.094	2.105	2.115	2.124	2.134	2.144	2.154	2.164
184	2.029	2.040	2.051	2.061	2.072	2.083	2.093	2.103	2.113	2.123	2.133	2.143	2.153	2.163	2.173
185	2.037	2.048	2.059	2.069	2.080	2.091	2.101	2.111	2.121	2.131	2.141	2.152	2.162	2.171	2.181
186	2.045	2.056	2.067	2.077	2.088	2.099	2.109	2.119	2.130	2.140	2.150	2.160	2.170	2.180	2.190
187	2.053	2.064	2.075	2.085	2.096	2.107	2.117	2.127	2.138	2.148	2.158	2.168	2.178	2.188	2.198
188	2.061	2.072	2.083	2.094	2.104	2.115	2.126	2.136	2.146	2.157	2.167	2.177	2.187	2.197	2.207
189	2.069	2.080	2.091	2.102	2.112	2.123	2.134	2.144	2.155	2.165	2.175	2.186	2.196	2.206	2.216
190	2.077	2.088	2.099	2.110	2.120	2.131	2.142	2.152	2.163	2.173	2.183	2.194	2.204	2.214	2.224
191	2.085	2.096	2.107	2.118	2.128	2.139	2.150	2.160	2.171	2.181	2.191	2.202	2.212	2.222	2.232
192	2.093	2.104	2.115	2.126	2.136	2.147	2.158	2.169	2.179	2.190	2.200	2.211	2.221	2.231	2.241
193	2.101	2.112	2.123	2.134	2.145	2.156	2.167	2.177	2.188	2.199	2.209	2.219	2.229	2.240	2.250
194	2.109	2.120	2.131	2.142	2.153	2.164	2.175	2.185	2.196	2.207	2.217	2.227	2.238	2.248	2.258
195	2.116	2.127	2.139	2.150	2.161	2.172	2.183	2.193	2.204	2.215	2.225	2.236	2.246	2.256	2.266
196	2.124	2.135	2.147	2.158	2.169	2.180	2.191	2.201	2.212	2.223	2.233	2.244	2.254	2.265	2.275
197	2.132	2.143	2.155	2.166	2.177	2.188	2.199	2.210	2.220	2.231	2.241	2.252	2.263	2.273	2.283
198	2.140	2.151	2.163	2.174	2.185	2.196	2.207	2.218	2.228	2.239	2.250	2.260	2.271	2.281	2.291
199	2.148	2.159	2.171	2.182	2.193	2.204	2.215	2.226	2.237	2.248	2.258	2.269	2.279	2.290	2.300

Table VI

Surface Area in Square Meters (Du'Bois Formula). 95 kg. to 109 kg., 135 cm. to 199 cm.

	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109
135	1.744	1.752	1.760	1.767	1.775	1.782	1.790	1.798	1.805	1.812	1.820	1.827	1.834	1.842	1.849
136	1.753	1.761	1.769	1.777	1.784	1.792	1.799	1.807	1.814	1.822	1.829	1.837	1.844	1.851	1.859
137	1.763	1.771	1.778	1.786	1.793	1.801	1.809	1.817	1.824	1.831	1.839	1.847	1.854	1.861	1.869
138	1.772	1.780	1.788	1.796	1.803	1.811	1.818	1.826	1.834	1.841	1.849	1.857	1.864	1.871	1.878
139	1.781	1.789	1.797	1.805	1.813	1.821	1.828	1.836	1.843	1.851	1.859	1.866	1.874	1.881	1.888
140	1.790	1.798	1.806	1.814	1.822	1.830	1.837	1.845	1.853	1.860	1.868	1.876	1.883	1.891	1.898
141	1.800	1.808	1.816	1.824	1.831	1.840	1.847	1.855	1.863	1.870	1.878	1.886	1.893	1.901	1.908
142	1.809	1.817	1.825	1.833	1.841	1.849	1.857	1.865	1.872	1.880	1.888	1.895	1.903	1.910	1.918
143	1.818	1.826	1.834	1.842	1.850	1.858	1.866	1.874	1.881	1.889	1.897	1.905	1.912	1.920	1.928
144	1.827	1.836	1.844	1.852	1.860	1.868	1.875	1.884	1.891	1.899	1.907	1.915	1.922	1.930	1.937
145	1.837	1.845	1.853	1.861	1.869	1.877	1.885	1.893	1.901	1.909	1.916	1.924	1.932	1.940	1.947
146	1.846	1.854	1.863	1.871	1.879	1.887	1.895	1.903	1.910	1.918	1.926	1.934	1.942	1.949	1.957
147	1.855	1.863	1.872	1.880	1.888	1.896	1.904	1.912	1.920	1.928	1.936	1.944	1.951	1.959	1.967
148	1.864	1.873	1.881	1.889	1.897	1.905	1.913	1.921	1.929	1.937	1.945	1.953	1.961	1.969	1.977
149	1.873	1.882	1.890	1.898	1.906	1.915	1.923	1.931	1.939	1.947	1.955	1.963	1.971	1.979	1.986
150	1.882	1.891	1.899	1.908	1.916	1.924	1.932	1.940	1.948	1.956	1.964	1.972	1.980	1.988	1.996
151	1.891	1.900	1.909	1.917	1.925	1.933	1.941	1.949	1.957	1.966	1.974	1.982	1.990	1.998	2.006
152	1.900	1.909	1.918	1.926	1.934	1.942	1.950	1.959	1.967	1.975	1.983	1.991	1.999	2.007	2.015
153	1.909	1.918	1.927	1.935	1.943	1.952	1.960	1.968	1.976	1.984	1.993	2.001	2.009	2.017	2.024
154	1.918	1.927	1.936	1.944	1.952	1.961	1.969	1.977	1.985	1.993	2.002	2.010	2.018	2.026	2.034
155	1.927	1.936	1.945	1.953	1.962	1.970	1.978	1.987	1.995	2.003	2.011	2.020	2.028	2.036	2.044
156	1.936	1.945	1.954	1.962	1.971	1.979	1.987	1.996	2.004	2.012	2.021	2.029	2.037	2.045	2.053
157	1.945	1.954	1.963	1.972	1.980	1.988	1.997	2.005	2.013	2.022	2.030	2.038	2.046	2.054	2.063
158	1.955	1.963	1.972	1.981	1.989	1.998	2.006	2.015	2.023	2.031	2.040	2.048	2.056	2.064	2.073
159	1.964	1.972	1.981	1.990	1.998	2.007	2.015	2.024	2.032	2.040	2.049	2.057	2.065	2.073	2.082
160	1.973	1.981	1.990	1.999	2.007	2.016	2.024	2.033	2.042	2.050	2.059	2.067	2.075	2.083	2.091
161	1.981	1.990	1.999	2.008	2.016	2.025	2.033	2.042	2.051	2.059	2.068	2.076	2.084	2.092	2.100
162	1.990	1.999	2.008	2.017	2.025	2.034	2.043	2.052	2.060	2.068	2.077	2.085	2.094	2.102	2.110
163	1.999	2.009	2.017	2.026	2.034	2.043	2.052	2.061	2.069	2.078	2.086	2.095	2.103	2.112	2.120
164	2.008	2.017	2.026	2.035	2.043	2.052	2.061	2.070	2.078	2.087	2.095	2.104	2.112	2.121	2.129

165	2.017	2.026	2.035	2.044	2.052	2.061	2.070	2.079	2.087	2.096	2.105	2.113	2.122	2.130	2.138
166	2.026	2.035	2.044	2.053	2.061	2.070	2.079	2.088	2.096	2.105	2.114	2.123	2.131	2.139	2.148
167	2.034	2.044	2.053	2.062	2.070	2.079	2.088	2.097	2.105	2.114	2.123	2.132	2.140	2.148	2.157
168	2.043	2.053	2.062	2.071	2.079	2.088	2.097	2.106	2.114	2.123	2.132	2.141	2.149	2.158	2.166
169	2.052	2.062	2.071	2.080	2.088	2.097	2.106	2.115	2.124	2.132	2.141	2.150	2.159	2.167	2.176
170	2.061	2.070	2.079	2.088	2.097	2.106	2.115	2.124	2.133	2.141	2.150	2.159	2.168	2.176	2.185
171	2.070	2.079	2.088	2.098	2.106	2.115	2.124	2.133	2.142	2.151	2.160	2.169	2.177	2.186	2.194
172	2.079	2.088	2.097	2.106	2.115	2.124	2.133	2.142	2.151	2.160	2.169	2.178	2.187	2.195	2.204
173	2.087	2.097	2.106	2.115	2.124	2.133	2.142	2.151	2.160	2.169	2.178	2.187	2.196	2.205	2.214
174	2.096	2.106	2.115	2.124	2.133	2.142	2.151	2.160	2.169	2.178	2.187	2.196	2.205	2.214	2.223
175	2.105	2.114	2.124	2.133	2.142	2.151	2.160	2.169	2.178	2.187	2.196	2.205	2.214	2.223	2.232
176	2.113	2.123	2.133	2.142	2.151	2.160	2.169	2.178	2.187	2.196	2.205	2.214	2.223	2.232	2.241
177	2.122	2.132	2.142	2.151	2.160	2.169	2.178	2.187	2.196	2.205	2.215	2.224	2.233	2.241	2.250
178	2.131	2.141	2.151	2.160	2.169	2.178	2.187	2.197	2.206	2.215	2.224	2.233	2.242	2.251	2.260
179	2.140	2.150	2.159	2.169	2.178	2.187	2.196	2.206	2.215	2.224	2.233	2.242	2.251	2.260	2.269
180	2.148	2.158	2.167	2.177	2.186	2.195	2.205	2.214	2.223	2.232	2.242	2.251	2.260	2.269	2.278
181	2.157	2.167	2.176	2.186	2.195	2.204	2.214	2.223	2.232	2.241	2.251	2.260	2.269	2.278	2.287
182	2.166	2.176	2.185	2.195	2.204	2.213	2.223	2.232	2.241	2.250	2.260	2.269	2.278	2.287	2.296
183	2.174	2.184	2.193	2.203	2.212	2.222	2.231	2.241	2.250	2.259	2.269	2.278	2.287	2.296	2.305
184	2.183	2.193	2.202	2.212	2.221	2.231	2.240	2.250	2.259	2.268	2.278	2.287	2.296	2.305	2.314
185	2.191	2.201	2.211	2.221	2.230	2.240	2.249	2.259	2.268	2.277	2.287	2.296	2.305	2.314	2.323
186	2.200	2.210	2.220	2.229	2.239	2.249	2.258	2.268	2.277	2.286	2.296	2.305	2.314	2.323	2.332
187	2.208	2.218	2.228	2.238	2.247	2.257	2.267	2.277	2.286	2.295	2.305	2.314	2.323	2.332	2.341
188	2.217	2.227	2.237	2.247	2.256	2.266	2.276	2.286	2.295	2.304	2.314	2.323	2.332	2.341	2.351
189	2.226	2.236	2.246	2.256	2.265	2.275	2.285	2.295	2.304	2.313	2.323	2.332	2.341	2.350	2.360
190	2.234	2.244	2.254	2.264	2.274	2.283	2.293	2.303	2.312	2.322	2.331	2.341	2.350	2.359	2.369
191	2.242	2.253	2.263	2.272	2.282	2.292	2.302	2.312	2.321	2.330	2.340	2.350	2.359	2.368	2.378
192	2.251	2.261	2.271	2.281	2.291	2.301	2.311	2.321	2.330	2.339	2.349	2.359	2.368	2.377	2.387
193	2.260	2.270	2.280	2.290	2.300	2.310	2.320	2.330	2.339	2.348	2.358	2.368	2.377	2.386	2.396
194	2.268	2.279	2.289	2.299	2.308	2.318	2.328	2.338	2.347	2.357	2.367	2.377	2.386	2.395	2.405
195	2.277	2.287	2.297	2.307	2.317	2.327	2.337	2.347	2.356	2.366	2.376	2.386	2.395	2.404	2.414
196	2.285	2.296	2.306	2.316	2.326	2.336	2.346	2.356	2.365	2.375	2.385	2.394	2.404	2.413	2.423
197	2.294	2.304	2.314	2.324	2.334	2.344	2.354	2.364	2.374	2.383	2.393	2.403	2.413	2.422	2.432
198	2.302	2.313	2.323	2.333	2.343	2.353	2.363	2.373	2.382	2.392	2.402	2.412	2.422	2.431	2.441
199	2.310	2.321	2.331	2.341	2.351	2.361	2.371	2.381	2.391	2.401	2.411	2.421	2.431	2.440	2.450

all, theoretically, should be correct to four places when accuracy to three places is desirable. However, the importance of these theoretic considerations is diminished by the fact that weight and height can be measured accurately to but three significant figures.

SUMMARY

Values of the Du Bois formula for human surface area are tabulated by unit cm. and kg. intervals for individuals of 110 to 200 cm. in height and of 20 to 110 kg. in weight.

Thanks are due George E. Redfern for the greater part of the routine calculation.

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STABILITY OF STANDARD KAHN ANTIGEN*

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A PROBLEM of practical importance in connection with a serologic test for syphilis is the stability of the antigen used. Early in 1924 the method of preparing antigen for the Kahn test was so standardized as to assure an unusual degree of uniformity.[1] It was still to be determined how long the antigen thus prepared would continue to react with the same degree of sensitiveness as when made; whether this sensitiveness would be affected by the temperature of storage, and whether an antigen would deteriorate after cholesterinization. In an attempt to determine these points the following experiments were carried out.

EXPERIMENTAL

Effect of storage at various temperatures.—Given amounts of Kahn standard antigen were sealed in glass ampules and stored at each of the following temperatures: 0°, 21°, 37°, and 54° C.† At the end of one day, three days, one, two, five, nine and nineteen weeks, respectively, each of the four experimental antigens was tested for its sensitiveness with 20 serums of varying potency—employing the Kahn routine test. After each period of storage, the antigens at the different temperatures gave results comparable among themselves and to a freshly prepared standard antigen used as a control. When the results did not show absolute agreement, the variations were not more than one-plus except in two cases where there was a difference of two-plus. Quite obviously, such small variations may be considered as insignificant. Table I gives the findings after five, nine and nineteen weeks' storage. The results recorded are those obtained in the second tube of the routine test (0.025 c.c. antigen dilution plus 0.15 c.c. serum).

Effect of boiling.—Since the antigen was apparently unaffected at a temperature of 54° C., experiments were carried out to determine whether it was sufficiently stable to withstand boiling. Five sealed glass ampules of antigen were immersed in boiling water and removed at the end of one, five, fifteen, thirty and sixty minutes, respectively. These five antigens were then tested with serums of varying potency, using standard antigen, unheated, as a control and employing the second tube of the routine test (0.025 c.c. antigen dilution plus 0.15 c.c. serum). The results indicated that the variations in the reactions given by the antigens kept at the boiling temperature of water for various periods, as compared with unheated control antigen were so slight as to be practically insignificant. Table II gives the findings with 40 serums.

Results with antigens stored for a year.—In the first experiment, the findings (Table I) indicated that the results with standard antigen after a period of nineteen weeks' storage were practically the same as those given

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†The antigen stored at 0° C. contained a precipitate which redissolved when the ampule was placed in warm water for a few minutes.

by the control antigen which was freshly prepared. In order to observe the effect of storage for a still longer period, comparative tests with serums of varying potency were carried out, using a standard antigen kept at 21° C. for a year, and one prepared a week before its use in the tests. Three different old antigens were used—antigen A in series 1, antigen B in series 2 and antigen C in series 3. The results with each of the old antigens were comparable with those obtained with a freshly prepared antigen. The few slight variations were such as might be expected with two different antigens. Table III gives the findings with 170 serums. Each finding is the average of the results obtained in the three tubes of the routine test.

Age and Cholesterinization.—An alcoholic extract which had been stored a year was cholesterinized a day before its use and tested with serums in comparison with the same lot of alcoholic extract cholesterinized immediately after its preparation and a week before its use in the tests. The findings with both antigens, as well as with a freshly prepared antigen, were practically

TABLE I

RESULTS WITH ANTIGEN AFTER VARIOUS PERIODS OF STORAGE AT DIFFERENT TEMPERATURES

PERIOD OF STORAGE OF ANTIGEN	NUMBER OF SERUMS	STANDARD ANTIGEN CONTROL	STORAGE TEMPERATURES			
			0°C.	21°C.	37°C.	54°C.
5 weeks	3		++++	++++	++++	++++
	6		+++	+++	+++	+++
	1		++	++	+++	++
	4		++	++	++	+
	2		++	++	+	−
	1		+	+	+	+
	1		−	+	−	−
	2		−	−	−	−
9 weeks	4	++++	++++	++++	++++	++++
	3	++++	++++	++++	++++	++++
	2	++++	++++	++++	++++	++++
	2	++	++	++	++	+
	2	+	+	+	−	−
	7	−	−	−	−	−
19 weeks	1	++++	++++	+++	+++	+++
	4	+++	+++	+++	+++	+++
	1	+++	++++	+++	++	++
	2	+++	++	++	++	++
	3	++	++	++	++	++
	1	++	++	+	+	+
	2	+	+	+	+	+
	6	−	−	−	−	−

TABLE II

RESULTS WITH ANTIGEN AFTER VARYING PERIODS IN A BOILING WATER-BATH

NUMBER OF SERUMS	STANDARD ANTIGEN (CONTROL)	PERIODS OF BOILING				
		1 MIN.	5 MIN.	15 MIN.	30 MIN.	60 MIN.
9	++++	++++	++++	++++	++++	++++
2	++++	++++	+++	+++	+++	+++
4	+++	+++	+++	+++	+++	+++
1	++	++	++	++	++	++
1	++	+	++	+	+	+
1	++	+	+	+	+	+
1	+	+	+	++	+	++
2	+	+	+	+	+	+
19	−	−	−	−	−	−

TABLE III

RESULTS WITH ANTIGENS STORED FOR ONE YEAR

SERIES 1			SERIES 2			SERIES 3		
NO. OF SERUMS	NEW ANT. CONTROL	OLD ANT. A	NO. OF SERUMS	NEW ANT. CONTROL	OLD ANT. B	NO. OF SERUMS	NEW ANT. CONTROL	OLD ANT. C
19	++++	++++	14	++++	++++	19	++++	++++
5	++++	+++	2	++++	+++	3	+++	++++
1	++++	++	2	+++	++++	6	+++	+++
1	+++	+++	9	+++	+++	1	++	++++
2	+++	++	3	+++	++	3	++	+++
1	+++	+	1	++	+++	9	++	++
2	++	++	2	++	++	4	+	++
5	++	+	1	+	++	1	+	+
9	+	+	8	+	+	2	±	+
15	−	−	8	−	−	12	−	−

TABLE IV

RESULTS WITH NEWLY CHOLESTERINIZED ANTIGEN COMPARED WITH OLD CHOLESTERINIZED ANTIGEN

NO. OF SERUMS	DATE OF PREPARATION OF ALCOHOLIC EXTRACT		
	MARCH 20, 1925	MARCH 1, 1924	
	DATE OF CHOLESTERINIZATION		
	MARCH 21, 1925	MARCH 2, 1924	MARCH 21, 1925
11	++++	++++	++++
1	++++	++++	+++
1	++++	+++	++++
3	++++	+++	+++
2	+++	+++	+++
1	+++	++	++
2	++	++	++
2	++	+	+
2	+	+	+
15	−	−	−

Date of tests: March 27, 1925.

in agreement. Table IV shows the results with 40 serums. Each finding is the average of the results obtained in the three tubes of the routine test.

SUMMARY

Kahn standard antigen was found to be practically unaffected by storage at 0°, 21°, 37° and 54° C. up to a period of nineteen weeks. It withstood the temperature of boiling water for at least an hour. After storage at 21° C. for one year, three different antigens gave results comparable to those given by freshly prepared antigen. An antigen stored for a year after cholesterinization gave practically the same results as the alcoholic extract stored for the same length of time but cholesterinized just before its use.

CONCLUSION

Our findings indicate that Kahn antigen is a relatively stable product. From a practical standpoint, the findings suggest that Kahn antigen can be stored safely at room temperature (in the dark) for long periods; it is unaffected by temperature changes accompanying distribution through the mails and frequent cholesterinization of the alcoholic extract is unnecessary.

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LABORATORY METHODS

AN OUTFIT FOR THE COLLECTION OF SERUM BY PHYSICIANS FOR THE DARK-FIELD EXAMINATION IN SUSPECTED SYPHILIS*

By William Royal Stokes, M.D., and C. Leroy Ewing, Baltimore, Md.

THE importance of an early diagnosis of syphilis is so apparent that this fact hardly warrants any further comment, since it has been sufficiently emphasized by a large number of authors. Some of these authors point out that the Treponema pallidum can often be demonstrated in the secretions taken from any open lesion as well as from the primary lesion. It has also been detected in the secondary rash and from the inguinal glands. It is important, therefore, to make an early diagnosis from the primary lesion if possible, by a microscopic examination of the serum obtained from such a lesion; if this method is extensively practiced, a much larger percentage of permanent cures can be effected. It is often the unrecognized and neglected cases originating from an atypical papule, herpes, or other such lesion, which are the most stubborn, and which develop the chronic lesions of the circulation, abdominal viscera, or nervous system, which exact the high toll and final mortality of syphilis.

Newsholme[1] quotes Osler's statement that syphilis is more destructive to life than tuberculosis, cancer, or pneumonia, although this comparison is based on an estimate of the deaths from syphilis listed under such causes as deaths of the insane, heart disease, paralysis, cerebral hemorrhage, premature birth, cancer of the tongue, and other conditions, and do not appear in the vital statistics listed as syphilis deaths. Newsholme attempted to "get nearer the truth regarding syphilis as a cause of mortality," and his table on page 484 shows a death rate ascribable to syphilis of 119.5 per 100,000 of the population.

It is therefore important to make an early diagnosis in as many cases as possible. Without early diagnosis, we can scarcely hope to effect any large reduction in syphilis mortality. Our Health Department, in keeping with the general custom of other departments, maintains a number of clinics in which the dark-field examination is made. Many cases of syphilis are detected in the early stage of the disease at these clinics and the proper treatment instituted. In addition, the Bureau of Bacteriology makes a dark-field examination from the primary lesion of any patient sent directly to the laboratory with a note from his physician requesting such an examination. Comparatively few patients, however, come to the Bureau for such examinations, and the laboratory, therefore, has recently devised an outfit in which physicians

*From the Bureau of Bacteriology, Health Department, Baltimore, Md.
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can obtain dark-field specimens in their offices, the outfit being sent to the Health Department for examination. This method overcomes the objections which many patients have against appearing in person at the Health Department for examination, and it maintains the due privacy which a physician may wish to preserve for his patients.

Each outfit contains a fine pipette with a rubber nipple. The serum is obtained either by capillary attraction or by a slight pressure upon the nipple. After the collection of the specimen, the pipette is placed within a glass tube and is sent to the laboratory as soon as possible, as our experiments have demonstrated that the motility often becomes sluggish about two hours after collection. In a circular sent out in the monthly Health News, the physicians have been urgently requested to use this outfit. A physician can obtain it at any one of about 160 culture stations distributed throughout the city or at the Health Department.

If a negative report is received, the physicians are then urged to send their patient to the Health Department for another examination, and the view is expressed in the circular that "if the physicians will cooperate with the Department in detecting these early cases of syphilis, it is felt that many cases will be saved from those future developments which may eventually destroy the life of the patient. By carrying out this technic, it is hoped that the mortality from the organic lesions due to syphilis may be gradually reduced."

EXPERIMENTAL WORK

Schereschewsky[2] has performed some experiments which should be considered in connection with this outfit. He uses a capillary pipette with a rubber nipple to scrape away the superficial covering of the lesion, and then uses the pipette to place four drops of normal salt solution over the lesion, and mixes this salt solution with the serum that exudes from the surface. He then uses a U-shaped capillary tube open at both ends, with one side

TABLE I

TABLE SHOWING DURATION OF MOTILITY OF TREPONEMA PALLIDUM IN POSITIVE CASES

TYPE OF LESION	LENGTH OF TIME BETWEEN COLLECTION AND DARK-FIELD EXAMINATION	CHARACTER OF MOTILITY	TEMPERATURE OF INCUBATION
Chancre	5 hours	Wavy and side to side movement	85° F.
Chancre	5 "	Active, wavy, and side to side movement	80° F.
Chancre	2 "	Slight side to side movement	98° F.
Chancre	2 "	Slight side to side and wavy movement	68° F.
Chancre	5 "	Slight side to side and wavy movement	70° F.
Chancre	24 "	Motility absent	70° F.
Chancre	2 "	Slight side to side and wavy movement	70° F.
Chancre	2 "	Slight side to side and wavy movement	70° F.
Chancre	5 "	Motility absent	70° F.
Chancre	8 "	Active, wavy, and side to side movement	70° F.
Mucous Patch	4 "	Active, wavy, and side to side movement	80° F.

having a diameter three times greater than the other, the serum being drawn up into the finer capillary end by capillary attraction. This capillary tube is detached from the other tube and sealed at both ends, and can then be transported from a clinic to the laboratory for examination. He states that he has observed motility in the Treponema pallidum for three weeks, when preserved in such closed tubes. This method, therefore, might be easily used for sending specimens by mail.

Willett,[3] of the St. Louis Health Department, in extragenital lesions which are not readily accessible, has used a capillary pipette with a rubber nipple to advantage.

Fig. 1.—Shows two formalinized specimens of the Treponema pallidum. (Mr. Hermann Becker.)

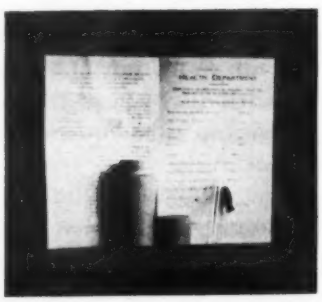

Fig. 2.—Photograph of the outfit for the collection of serum described in the text. (Mr. T. C. Buck.)

In obtaining specimens in the city, however, we have found the use of the second U-tube unnecessary, and have simply collected pure serum in the pipettes by the method which will be described below in detail.

These specimens have been collected from patients who reported to the Health Department or to some of our neighboring clinics. The table shows the time which intervened between the collection and the examination, including the variety or loss of motility after different periods of time.

The table shows that most of our specimens were active after five hours, but, as one was negative at the end of this time, we would suggest that no greater interval than four hours elapse between the collection of the specimen

and the dark-field examination. These specimens were all taken from cases which were demonstrated as positive immediately before the use of the tube.

APPLICATION TO TEACHING STUDENTS

We have also been able to use our method in demonstrating to students the appearance of the Treponema pallidum. By collecting a specimen at the clinic, we have been able to use it later in several dark-field apparatus and to demonstrate to medical students, at the University of Maryland, the appearance and activity of the organism. By mixing an equal quantity of a 10 per cent solution of formalin with several drops of the serum and spreading it out between a slide and cover slip, we have preserved such specimens for several months. This is accomplished by carefully sealing the sides of the cover slip with balsam in order to prevent evaporation. The student first becomes familiar with the appearance of the formalinized organism under the dark-field, and then later examines motile, active specimens obtained from the clinic. We consider these demonstrations a definite part of the crusade against the mortality from syphilis, since with more medical men familiar with the organism, a larger number of early diagnoses will be made.

Fig. 1 is a photomicrograph of the Treponema pallidum preserved for class instruction, which also shows the appearance of a spirochete which has been obtained from an active lesion and which has lost its motility. Even under this condition, we consider the appearance of such a specimen just as characteristic as that of a fixed spirochete which has been stained by any of the ordinary staining methods.

DESCRIPTION OF TECHNIC

The technic of collecting the specimen is described in the circular which accompanies all of our outfits. Fig. 2 shows a photograph of this outfit.

INSTRUCTIONS FOR OBTAINING A SPECIMEN FOR DARK-FIELD EXAMINATION FROM A PRIMARY LESION IN SUSPECTED SYPHILIS.

The specimen should be collected preferably with the patient lying on a couch. The lesion is thoroughly washed and sponged with warm tap water, using absorbent cotton. The physician should rub the lesion with a sterile gauze sponge until bleeding is produced, and this can only be aided by a moderate amount of pressure. It is important to remove the superficial epithelium in order to reach the deep portion of the lesion. The blood should be wiped away, and nothing further should be done until the lesion is bathed in serum. A curette may be used instead of gauze.

The operator then applies the open tip of the pipette to the serum, and sucks up enough serum by means of the nipple until the fluid reaches the even bore of the tube, as indicated by the red line. The serum should be as free from blood as possible.

The pipette should then be replaced very lightly in the small glass test tube and not forced in, and great care should be taken not to expel the serum from the capillary pipette into the test tube. The specimen should then be brought at once to the laboratory during laboratory hours, and delivered personally to one of the bacteriologists, and no specimens will be accepted after 4 o'clock on week days, after 11 o'clock on Saturdays, or on Sundays or holidays.

The operator should always wear rubber gloves, which should be sterilized after the specimen has been obtained.

If a patient has been applying any treatment to the lesion, especially if any preparation of mercury is used, this may influence the result of the examination, producing a false negative reaction. Under such conditions the patient should be instructed to discontinue such treatment and to wash the lesion thoroughly with soap and warm water two or three times a day and to report back for examination on the second day.

In all cases of a negative result the patient should be sent to the bacteriological laboratory at eleven o'clock on week days for examination.

Please fill out the data requested on the reverse side of the blank.

The reverse side of the instructions contains the data necessary for the notification of the physician and the laboratory record.

Laboratory No._____

HEALTH DEPARTMENT

DARK-FIELD EXAMINATION OF SPECIMEN FROM THE PRIMARY LESION IN SUSPECTED SYPHILIS

See directions for collecting specimen on other side.

Have previous specimens been sent?		Number
Date of taking specimen		
Name of patient		Age
Address		Sex
Occupation		Color
Married	Single	Wid.
Duration of disease dating from initial lesion		
Has salvarsan been administered?		When
Has mercury been administered?		When
Has any local treatment been applied?		
Date of last application		
Name of physician		Phone No.
Address		
Result of examination		

CONCLUSIONS

We believe that, if some such method as we have outlined above is adopted by city health departments, a much larger number of dark-field examinations can be made and early treatment thus instituted. Such a diagnosis will certainly aid in obtaining a permanent cure in a larger number of cases and thus help to reduce the mortality from syphilis. The method of the sealed tube of Schereschewsky can probably be used for sending specimens in an outfit through the mail, in the proper legal mailing outfit permitted by the post-office department. At least we believe that any method which may help to reduce the mortality from syphilis should be thoroughly tried out.

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BLOOD URIC ACID

Comparative Results by Three Methods, and Technic Necessary for Accurate Estimation*

By William P. Holbrook, B.S., and Howard D. Haskins, M.D.,
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ACCURATE estimation of uric acid in blood has been a baffling problem. The first step toward solving this problem was taken when Folin and Denis,[1] in 1912, proposed their phosphotungstic reagent for a microcolorimetric method and suggested precipitation of the uric acid from protein-free filtrate prepared from blood. Since that time, many other reagents for developing a color with uric acid have been offered. During the last three years we have tried those that seemed promising. None of them are superior to the Folin reagent in our judgment.

Our object in undertaking work on uric acid was twofold. First, to make a comparison of present methods and arrive at a simple accurate method for its estimation in blood. Second, to form a definite opinion as to the clinical value and necessity of estimating uric acid as an indication of kidney impairment. This paper will deal only with the first problem, as work on the second is still in progress.

After some preliminary work we selected three representative methods for investigation:

(1) The zinc chloride precipitation method suggested by Morris and Macleod,[2] (2) the silver lactate precipitation method of Folin and Wu,[3] and (3) the direct method first suggested by Benedict,[4] and later accepted by Folin.[5] All estimations were made on the Folin filtrate which is a 1 in 10 dilution of blood, freed of protein by tungstic acid. Folin's uric acid reagent and sodium cyanide solution were used for all three methods. The technic will be described later.

Our experimental work was divided into three parts, as follows:

(1) Estimations on accurate uric acid solutions prepared by weighing pure uric acid.

(2) Estimations on blood from 55 normal persons by three methods and a modification of each, making six determinations.

(3) Estimations on blood from 25 hospital cases by three methods.

After estimating a large number of accurate uric acid solutions by the three methods, following the original technic, we drew the following conclusions:

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(1) The results by the zinc method were invariably too low.
(2) The silver and the direct methods gave results that were accurate only when the concentration was 4 mg. or more per 100 c.c. of blood (using a standard equivalent to 4 mg. per 100 c.c.).
(3) Few of the estimations, by any method, were accurate when the concentration was 1 to 3 milligrams.

By changing the amounts of zinc chloride and sodium carbonate used, we were able to secure results by the zinc method that checked with those by the other two methods when the concentration was 4 mg.

We tried adding pure uric acid to the blood unknown just before adding the uric acid reagent and cyanide. When 5 c.c. of solution was added, containing the same amount of uric acid as the standard, the increase in volume spoiled the estimation (color unsatisfactory). We next reduced the amount of added uric acid to 0.5 c.c., using a more concentrated solution which contained five times as much uric acid as Folin's dilute standard. This addition was equivalent to an increase of 2 mg. per 100 c.c. of the original blood.

By such addition of uric acid to the unknown, we secured accurate results by all three methods, with solutions corresponding to blood specimens containing 1 to 6 mg. per 100 c.c.

Having modified three methods so that they checked accurately on pure uric acid solutions, we were ready to try them on normal blood samples. It was thought best to try each method with and without the addition of uric acid to the unknown making six estimations on each sample of blood. A summary of the accuracy of the results is given in Table I. It will be seen at a glance that by far the greatest accuracy is secured when uric acid is added to the unknown (our modification). Estimations by methods Nos. 2, 4, and 6 show this clearly. Over half of the estimations by the unmodified direct method (No. 5) were off color, making color comparison almost impossible. What causes this, we do not know. However, the addition of uric acid to the unknown (No. 6) certainly makes it easy to match the colors, so that the results are excellent. Similar good results were obtained by the modified precipitation methods (Nos. 2 and 4).

The third part of the experimental work was on blood from hospital

TABLE I

COMPARATIVE ACCURACY OF 6 URIC ACID METHODS ON 55 NORMAL BLOOD SPECIMENS

METHOD NUMBER	1	2	3	4	5	6
Number of estimations:						
Accurate	18	52	16	55	3	48
Excess	2	0	20	0	9	2
Slight excess	0	0	14	0	0	3
Slight deficiency	29	3	0	0	0	2
Off color, poor estimations	3	0	4	0	14	0
Total specimens	52	55	54	55	26*	55

Range of normals: 1.5 to 3.7 mg. uric acid per 100 c.c. blood.
Average of normals: 2.4 mg.

*This method was discontinued because the type of color made the estimation unsatisfactory.

Methods: Zinc method without added uric acid, No. 1, and with added uric acid, No. 2.
Silver method without added uric acid, No. 3, and with added uric acid No. 4.
Direct method without added uric acid, No. 5, and with added uric acid, No. 6.

TABLE II

COMPARISON OF RESULTS BY THREE MODIFIED METHODS (URIC ACID ADDED) ON HOSPITAL BLOOD SPECIMENS

METHOD	MODIFIED ZINC (NO. 2) MG.	MODIFIED SILVER (NO. 4) MG.	MODIFIED DIRECT (NO. 6) MG.	VARIATION NO. 2 FROM NO. 4 MG.	VARIATION NO. 6 FROM NO. 4 MG.
Case 1	0.8	0.9	0.8	0.1	0.1
2	1.1	1.1	1.0	0.0	0.1
3	1.7	1.6	1.7	0.1	0.1
4	2.0	2.1	2.0	0.1	0.1
5	2.0	2.1	2.0	0.1	0.1
6	2.0	2.1	1.9	0.1	0.2
7	2.4	2.5	2.5	0.1	0.0
8	2.5	2.6	2.6	0.1	0.0
9	2.6	2.6	2.6	0.0	0.0
10	3.0	3.0	3.1	0.0	0.1
11	3.5	3.6	3.6	0.1	0.0
12	3.5	3.6	4.0	0.1	0.4
13	3.5	3.7	3.7	0.2	0.0
14	3.7	3.7	3.7	0.0	0.0
15	4.1	4.0	4.2	0.1	0.2
16	4.1	4.4	4.4	0.3	0.0
17	4.2	4.2	4.4	0.0	0.2
18	5.7	5.7	6.0	0.0	0.3
19	10.0	10.3	10.2	0.3	0.1
20	10.2	10.2	10.2	0.0	0.0
21	10.6	10.7	10.7	0.1	0.0
22	11.4	11.4	11.2	0.0	0.2
23	13.0	13.1	13.1	0.1	0.0
24	13.1	13.2	13.3	0.1	0.1
25*	13.3	13.1	13.3	0.2	0.2
Average Variation				0.09	0.1

*Uric acid not added (methods 1,3 and 5 used).

cases. Because of the superiority of Methods 2, 4, and 6 as shown by the previous work on normals, we decided to use only these three methods. An attempt was made to select cases in which uric acid findings might be significant. The estimations on 25 cases and their variations are given in Table II. For purposes of comparison the estimation by the silver method was taken as standard, and the variation from it was computed. The results show that all three methods are quite satisfactory from the standpoint of accuracy.

TECHNIC

Direct Estimation Method (Modified Folin-Benedict).—Measure exactly 5 c.c. of blood filtrate and 0.5 c.c. of modified standard uric acid solution into a test tube. Into a second tube measure 1 c.c. modified standard uric acid and 4.5 c.c. of water. Now add to each of the tubes 3 drops of 20 per cent lithium sulphate, exactly 1 c.c. of uric acid reagent, and finally, just 2 c.c. of sodium cyanide solution. Mix and, after standing two minutes, heat in a boiling bath 70 to 80 seconds. Cool in water to room temperature, dilute to 25 c.c., and mix. Estimate at once setting the standard at 20 mm.

Calculation: $\left(\frac{20 \times 4}{\text{mm. unknown}}\right)$ minus 2 = mg. uric acid in 100 c.c. blood.

Zinc Chloride Check Method (modified Morris and Macleod).—To 5 c.c. of blood filtrate in a centrifuge tube add 2 c.c. of water, exactly 0.4 c.c. of 1 per cent zinc chloride solution, and 0.7 c.c. of 2 per cent sodium carbonate solution (made from anhydrous carbonate). Stir with a rod, then rinse the

rod with a few drops of water. Balance with another tube. After standing five minutes, centrifuge for three minutes. Pour off the clear liquid (not used). To the precipitate add 1 c.c. of Folin's HCl-NaCl (10 gm. sodium chloride dissolved in 100 c.c. of tenth-normal HCl) and stir with a rod. Dilute with 4 c.c. of water and stir until all is dissolved. Rinse the rod. Pour the liquid (drain fully) into Tube I. Also add 0.5 c.c. of modified standard uric acid solution. Into Tube II measure 4.5 c.c. of water and 1 c.c. of modified uric standard. Into each tube put three drops of 20 per cent lithium sulphate, 1 c.c. of uric reagent, and 2 c.c. of sodium cyanide. Mix, after two minutes heat in the bath for 80 seconds, cool, dilute to 25 c.c., and mix. Estimate with the colorimeter and calculate as in the direct method.

Reagents.—

(1) Folin's sodium cyanide solution: Dissolve 15 gm. of purest cyanide in 100 c.c. of tenth-normal NaOH. Impurities cause the fresh solution to give considerable blue color with the uric acid reagent, interfering with the estimation. After two weeks, the impurity gives little color. When four months old, it should be discarded or else purified by aeration to remove ammonia. Very old solutions give an improper blue color.

(2) Folin's standard uric acid solution. (a) Strong stock solution: dissolve 0.5 gm. of pure uric acid and 0.25 gm. of lithium carbonate in 75 c.c. of hot water (60°) in a 500 c.c. flask. When dissolved clear, cool and add 175 c.c. of water and 12.5 c.c. of 40 per cent formalin. Shake, add 3 c.c. of glacial acetic acid, mix, and fill to the mark. This solution keeps for a very long time. (b) Modified dilute standard: mix 2 c.c. of stock solution, 50 c.c. of water, 5 c.c. of two-thirds normal sulphuric acid and 0.5 c.c. of formalin in a 100 c.c. flask and fill to the mark. This is five times as strong as the dilute standard used by Folin. Make the solution every two months.

(3) Folin's original uric acid reagent: Dissolve 50 gm. of C. P. sodium tungstate in 350 c.c. of water; add 40 c.c. of 85 per cent phosphoric acid, and heat the flask, having a reflux tube attached. Boil for two hours, cool, and dilute to 500 c.c.

(4) One per cent zinc chloride solution. When first made the solution is likely to be cloudy, but it is perfectly satisfactory when filtered.

DISCUSSION

Although half of our hospital cases fail to show uric acid retention, all were selected on account of the possibility of increase of uric acid content of the blood. They included cases of nephritis (of various types), prostatic obstruction, arthritis, gout, hypertension, and leucemia. A complete blood chemistry examination was made in almost every case. The report of the results and the discussion of their relation to uric acid retention are reserved for a future paper.

We recommend the zinc method for use whenever it is necessary to make a check on the results by the direct method, since our modifications have made the method reliable. It is much cheaper and a little quicker than the silver lactate method.

Estimations by the silver method were reported by Rogers[6] to be inaccurate because of a rapid change due to exposure to light. Our work does

not confirm this. Our results have been practically identical when an estimation exposed to ordinary diffused daylight for fifteen minutes was compared with one run in the dark.

It is quite possible that an occasional clinical blood specimen will give too high an estimation by the direct method because of the presence of certain substances other than uric that give a blue color with the reagent. Lennox and O'Connor,[7] also Bulmer, Eagles, and Hunter[8] reported a number of blood samples showing large variations in results by the precipitation and the direct methods. Others have secured excess estimations on animal blood by the direct method. Of the 80 specimens examined by us only one (Case 12) showed a distinct variation (10 per cent).

Folin[9] has recently put out an improvement of his uric acid reagent. This has been freed of molybdenum (present as an impurity) and is supposed to give the blue color only with uric acid. It is to be hoped that this reagent will make the direct estimation reliable in the case of all blood specimens. In the series of estimations which we have made by the modified direct method (No. 6), using both the original and the improved reagent, the results were identical. However, when the unmodified direct method is used, the new reagent gives a better type of color than does the old reagent. The preparation of the improved reagent is rather tedious. We believe that in all but a very small percentage of cases, the old reagent is quite satisfactory if our modified technic is followed.

While it is true that the addition of uric acid to the unknown is not necessary for a uric acid content above 6 mg., yet, estimations made with added uric acid are satisfactory at all levels.

CONCLUSIONS

1. For estimating blood specimens that have a normal uric acid content, our suggestion of adding uric acid to the unknown must be followed to secure sufficient color and a proper type of color for accurate estimation.

2. The direct method, which has previously been very unsatisfactory, is now reliable in most cases, provided our technic of adding uric acid to the unknown is followed.

3. The zinc precipitation method as modified by us has proved a very satisfactory check method, and the cheapness of the reagents as compared with those of the silver method, make it desirable. It is not necessary to use this method in preference to the direct method unless uric acid retention is indicated by the latter.

4. The normal uric acid content of the blood in 55 individuals varied from 1.5 to 3.7 mg. per 100 c.c., and averaged 2.4 mg.

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A METHOD FOR STAINING NERVE CELLS EN BLOCK WITH BASIC ANILINE DYES*

By Frederic Proescher, M.D., and Albert S. Arkush, A.B., Agnew, California

THE original Nissl method[1] for the elective staining of nerve cells is, in spite of its technical difficulties, still the method of choice. It aims to produce a standard nerve cell picture, regardless of the true morphologic structure of the nerve cell, as long as the conditions under which it is obtained are identical. The structures made visible may, or may not, be identical with the living organism. Nevertheless, the method furnishes a standard of comparison for the living cell structures.

Nissl has shown that 96 per cent alcohol is the fixative par excellence. According to Nissl, sections should be made from the unembedded material and stained in an aqueous solution of a basic aniline dye (methylene blue) followed by differentiation in aniline alcohol, etc.

The main difficulty in this method is the sectioning of the unembedded material. Sections of the pia and degenerated areas, as well as of whole hemispheres, are especially difficult to obtain. The staining and differentiation require a great deal of patience and skill in order to obtain satisfactory specimens. In fact, the entire process is difficult and comparatively limited from a technical standpoint.

To overcome these difficulties, celloidin and paraffin embedding and staining with toluidin blue, thionin, or cresyl violet is advocated,[2] but none of these combinations give specimens favorably comparable to those from unembedded material.

A large number of staining experiments with alcohol-fixed brain material have shown us that certain basic aniline dyes, in alcoholic solution and at various temperatures and ion concentrations, easily penetrate brain and spinal tissue blocks to practically any depth.

We have tried a large number of basic aniline dyes in alcoholic solution and find that methylene azur (thiazin red) and toluidin blue are the only stains of value. Neutral red, indulin, pyronin, nigrosin, cresyl violet, methylene blue, and methyl green have been tried without success. As a stain, we recommend methylene azur[3] which can be easily prepared in any desired quantity from methylene blue by oxidation with sodium peroxide. The crude methylene azur should be recrystallized, at least three times, from redistilled alcohol. The dyes are dissolved in alkalized chemically pure ethyl or methyl alcohol.

Recent investigations have demonstrated the importance of the ion concentration of staining solutions.[4] We adjusted the alcoholic solution to ion concentrations varying from P_H values of 7.4 to 12.

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The blocks, after removal from the staining solution, were uniformly stained a deep blue. The cortex is always of a deeper shade than the medullary substance, and stains much more rapidly. Some of the stain is removed by a short washing of the blocks in absolute methyl or ethyl alcohol. Immersion in xylol, which follows, stains the xylol bluish violet to violet, indicating that the free base of the dye is removed. If the xylol is changed until colorless, the blocks are almost translucent and of a deep bluish violet. The subsequent immersion of the blocks in molten paraffin removes no stain.

Microscopic examination of the sections shows that the nerve cells and especially the basophilic substance, including the nuclei, are electively stained, while the background is almost completely unstained. Other nonnervous elements are stained.

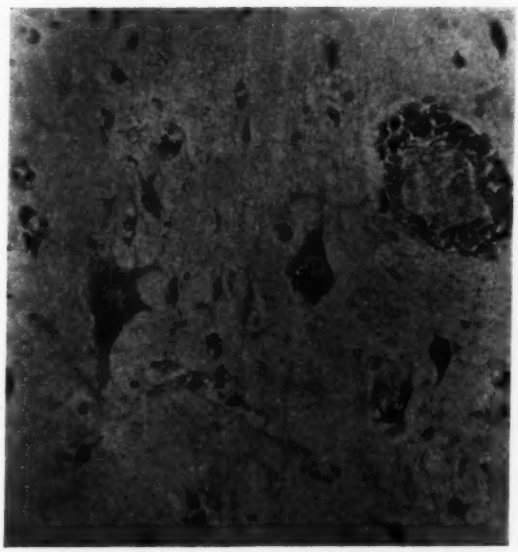

Fig. 1.—Section of paretic brain showing ganglion cells with basophilic substances, glia cells, and vessel with perivascular infiltration. Operative 4 mm., ocular No. 2.

Satisfactory penetration may be obtained for small blocks at room temperature or at 37° C. We find, however, that at 58 to 60 degrees, blocks of 4 to 8 mm. are well penetrated in three to five hours. Periods two to five times as long are required at the lower temperatures, depending on the size of the blocks.

While the alcoholic solution easily penetrates unaided, we find that the addition of glycerin adds both to the penetration of the stain and to the mechanical facility of sectioning. A range of two per cent to five per cent of glycerin is offered, as the penetration desired will vary with the type of work. Below two per cent there is an inappreciable effect and above 5 per cent the sections come off the microtome too soft and the staining is too diffuse. It seems that there is a definite relation, however, between the optimum amount of glycerin and the P_H of the staining solution (possibly due to impurities in the glycerin). Increase in alkalinity lowers the increased penetration due to glycerin, so that the higher value given above is recommended with high alkalinity.

The P_H values of 7.4 to 12 were found to give well differentiated sections. Seven and six-tenths to 10 are probably optimum limits. Outside these values the staining is either too weak or too heavy.

The staining results indicate that the basophilic substance of the nerve cells and certain nonnervous cellular elements can be electively stained, if xylol is used as the differentiating and clearing medium.

The question of whether or not the staining process is of a chemical or physicochemical nature, we leave undecided. Probably the basic elements of the nerve cells and other nonnervous elements form an insoluble dye salt with the stain, while the unstained nervous tissue has no affinity for the dye or forms a loose combination from which the stain is easily extracted.

A block has recently been stained by us which shows the protoplasmic and fibrous glia, as well as the basophilic substances of the nerve cells, stained and differentiated. This represents an almost ideal formula, and efforts are being made to determine how the result may be uniformly obtained. This particular block was stained for eleven and one-half hours at room temperature in a solution containing a small amount of eosin and a trace of toluidin blue, as well as methylene azur. It forms an excellently combined picture of the protoplasmic and fibrous glia and that of Nissl, for ganglion cells.

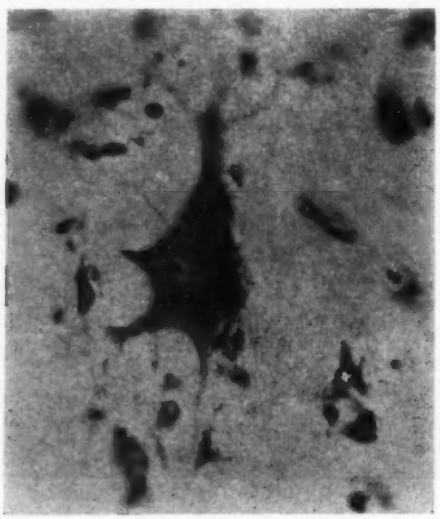

Fig. 2.—Ganglion cell shown in picture. 1. Oil immersion 1/12, ocular No. 2.

METHOD OF PROCEDURE

For preparing the staining solution, chemically pure methyl or ethyl alcohol is brought to the desired hydrogen-ion concentration by adding the sodium (or potassium) hydroxide as described. It will be found that 0.25 c.c. to 2.00 c.c. of the alcoholic alkaline solution approximately covers the useful P_H range. This may be compared with a colored buffer standard, if desired, but once determined for the character of work to be performed, such comparison will be found no longer necessary. Small changes, such as those introduced on addition of the dye (carefully prepared), are inconsequential.

1. To each 100 c.c. of the alkalized methyl or ethyl alcohol add 0.25 gm. of thiazin red.

2. Immerse the thoroughly fixed brain tissue (fixed in 96 per cent alcohol for at least five days) in the staining solution. This should be at least

two to three times the volume of the block. Keep here for five hours at a temperature of 56 to 60 degrees.

3. Remove the stained blocks and wash in methyl or ethyl alcohol. If glycerin is added to the staining solution, the alcohol should be renewed two or three times in ten to fifteen minutes to remove as much of the glycerin as possible and facilitate the penetration of xylol.

4. Immerse in xylol for three to four hours, changing the xylol at least three times.

5. Pass the block through xylol paraffin for several hours and then in molten paraffin for one to three hours.

6. Cut the block into thicknesses of 4 to 6 microns.

7. Mount the sections and dry them for several hours at 37° C. Glycerin egg-albumen should not be used in mounting if it is desired to keep the

Fig. 3.—Motoric ganglion cells from the anterior horn of a normal spinal cord. Objective 4 mm., ocular No. 2.

sections. In case it is used, care should be taken that the sections are thoroughly dried.

8. Melt the paraffin carefully over the flame, remove the paraffin with neutral xylol, and mount in xylol-colophonium.

With the above described staining method, an elective staining of the basophilic substances of nerve cells, including nuclei and nuclear membrane, glia cell bodies, and mesenchymal cellular elements of the vessel walls is possible. The neurofibrillae remain unstained, the fibrous glia either colorless or of a pale blue stain.

Compared with sections stained with the standard Nissl method, it seems that the protoplasmic structures, in general, are stained somewhat more deeply. The pigments are more distinct and certain nuclear elements, not shown by the original method, are stained metachromatically. This new factor can be added to the standard picture established by Nissl.

The following additional advantages of this method may be noted:

a) The cumbersomeness of the Nissl method is avoided.

b) Much thinner sections can be obtained and the finer details of the histologic changes of the nerve cells are more easily observed, and without precipitation of the dye.

c) Complete serial sections can be made.

d) Sections made by this method have been kept for six months without appreciable change. With the Nissl method, the majority of the sections deteriorate in short time.

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A METHOD OF BLOOD GROUPING WHERE ONLY ONE KNOWN GROUP IS AVAILABLE*

BY W. L. VOGEL, RIVERSIDE, CALIFORNIA

THE increasing frequency of blood transfusion has necessitated the development of simple laboratory methods for determining the suitability of donors, and has made blood grouping a procedure which the laboratory worker is frequently called upon to do. I have found the method herein outlined to be of greater convenience than the emergency practice of testing for crossagglutination when standard agglutinating sera of Groups II and III are not available. On the staff of every hospital or laboratory there is almost certain to be, at least, one individual known to possess a Group II blood. By the following technic we have found it possible to not only determine the suitability of a donor, but to group both donor and recipient.

TECHNIC

Secure 3 c.c. of blood from a Group II individual, from the prospective donor, and from the recipient. Allow one-half of each blood specimen to fall into three separate centrifuge tubes containing 1 per cent of sodium citrate in physiologic salt solution. Throw down the citrated cells, remove supernatant fluid with a pipette and wash the sediment with physiologic salt solution several times, repeating the procedure with the citrated half of each of the three blood specimens. In each instance, make a 10 per cent emulsion of the red cells in physiologic salt solution.

Remove the serum from the coagulated remainder of each of the three blood specimens, and clear by centrifuging.

*From the Clinical Laboratory, Riverside, California.
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Carry out the following procedure with the known Group II serum and cells against the recipient's serum and cells and against the prospective donor's serum and cells:

Into a small test tube (7 mm. by 10 cm.) pipette 0.2 c.c. of the Group II serum and 0.2 c.c. of the unknown cells and into a similar tube, pipette 0.2 c.c. of the unknown serum and 0.2 c.c. of the Group II cells. Mix the contents of each tube thoroughly by shaking and place in a water-bath at 37° C. for two hours. At the end of incubation, examine for agglutination.

INTERPRETATION OF RESULTS

If no agglutination occurs in either tube, the unknown belongs to Group II.

If agglutination occurs in both tubes, the unknown belongs to Group III.

If agglutination occurs only in the tube containing the unknown serum and the Group II cells, the unknown falls in Group IV.

If agglutination occurs only in the tube containing the Group II serum and the unknown cells, the unknown belongs to Group I.

TABLE I

	TUBE 1 (GROUP II SERUM AND UNKNOWN CELLS)	TUBE 2 (UNKNOWN SERUM AND GROUP II CELLS)
Group I	Agglutination	No Agglutination
Group II	No Agglutination	No Agglutination
Group III	Agglutination	Agglutination
Group IV	No Agglutination	Agglutination

While in our laboratory we prefer the macroscopic or tube method, using substantial quantities of serum and cells, the same method may be employed with much smaller quantities of blood secured from the puncture of finger or ear, the mixtures being made as hanging drop preparations on a concave microscope slide with two concavities, and examined after thirty minutes under the 16 mm. objective.

SUMMARY

A method of blood grouping is described requiring only such glassware and equipment as the clinician has available in his office for the microscopic examination of urinary sediment; where but a single blood specimen of a known group is at hand. Needless to say a similar technic may be evolved for use with a known Group III. It is suggested that the physician who is removed from laboratory facilities, have his own or the blood of the office assistant grouped, so that a test similar to the one outlined may be performed before emergency transfusion. In grouping blood specimens of the physician and office assistant in ten offices of general practitioners, one or more Group II or III persons were found in all but one office. Where laboratory service is at hand, we draw an additional 2 c.c. of blood from prospective donors for the routine Wassermann.

The principle of this technic was suggested to me by G. R. Rea, Professor of Bacteriology, University of Saskatchewan, Canada, and is gratefully acknowledged.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

Leveriza, J. C.: Anti-Allergic Treatment of Bronchial Asthma. Jour. Philippine Med. Assn., June, 1925.

The author advances the suggestion that the poor results often obtained in the treatment of bronchial asthma with autogenous vaccines from the sputum may be due to the absence in the vaccine of bacterial proteins and bacterial exogenous products to which the patient may be sensitized.

In order to complete the vaccine and to secure the complete bacterial flora of the respiratory tract together with a concentrate of the bacterial exogenous products, he prepares a vaccine reinforced with the sputum filtrate.

The sputum is first examined for the presence of B. tuberculosis and, if this organism is absent, cultures are made on Loeffler's blood serum without preliminary plating or attempts to isolate specific strains.

An equal volume of 0.9 per cent saline is then added to the sputum, the mixture thoroughly shaken and incubated with the sputum culture for twenty-four hours at 38° C. The sputum-saline mixture should be shaken occasionally. After twenty-four hours incubation the culture is examined for spore-bearers. If these are absent, a suspension is prepared and sterilized by heating for one hour at 60° C. as usual.

The saline-sputum mixture is then centrifuged and the supernatant fluid filtered through a Chamberland or Berkefeld filter and tested for sterility.

The sterile filtrate is used to dilute the vaccine suspension and 0.5 per cent phenol is added as a preservative.

The initial dose is small and increased at two-day intervals until a mild general or focal reaction occurs, this dose then being continued until a reaction fails to occur when the dose is again increased cautiously. The maximum dose (1 c.c.) is repeated at regular intervals for at least ten injections.

The method is admitted to be empirical and inexact but better results are claimed in true bronchial asthma than have been secured with the usual autogenous vaccines.

Fildes, P.: Isolation, Morphology, and Cultural Reactions of B. Tetani. Brit. Jour. Exper. Path., April, 1925, vi, 62.

The author reports a study of B. tetani conducted upon a large number of pure cultures obtained by the method described.

The usual laboratory media were used without the addition of glucose. To gelatin and Loeffler's medium 0.5 per cent of blood laked with an equal volume of distilled water was added.

Slanted solid media was not used until after the upper edge of the slant showed slight signs of drying, in order to bring out the characteristic edge of the film-like growth described below.

When the organisms were few in number a preliminary enrichment culture was made in laked-blood broth and incubated in air for from two to four days, the longer period being preferred.

From this enrichment culture two drops were inoculated into the water of condensation of a solid slant. The tubes were then incubated under anaerobic conditions secured by the use of the electrically-heated jar described by Fildes and McIntosh.

After twenty-four hours at 38° C. a spreading growth is seen on the surface of the slant, different bacteria showing "high-water" marks indicating the limits of growth.

B. tetani presents this ability to spread up the surface of the slant more markedly than other anerobes. When it is present, there will be seen above the highest watermark on the

apparently unaffected surface of the medium a fine, almost structureless film at times detected only by a hand lens. When the uppermost edge of the film encroaches on the drying edge of the medium there may be seen a fine tangle of tenuous filaments. If smears from this film show the presence of tetanus bacilli, a transplant made from the upper edge of the film to the water of condensation of a new slant generally gives a pure culture, a third tube being rarely required.

The only organism resisting this method of isolation is B. proteus which can be removed by heating the material prior to inoculation.

Studies were made of 75 strains thus isolated, with interesting observations on the morphology and cultural characteristics of the organisms.

The article is illustrated with 30 figures.

Boquet, A. and Negre, L.: Methylic Antigen in the Study of Tuberculous Antibodies and in the Treatment of Experimental Tuberculosis in Smaller Laboratory Animals. Presse Med., Paris, Oct. 3, 1925, lxxix, 1315.

The authors, from Calmette's laboratory, describe their attempts to prepare a "stable, unalterable, specific antigen" sufficiently sensitive to reveal all the fixing bodies in a tuberculous subject.

The method of preparation of the antigen is as follows:

Human and bovine tubercle bacilli are grown in glycerin broth for six weeks. The cultures are sterilized by exposure to 120° C. for thirty minutes, filtered through paper, and the bacterial residue mixed. The bacterial mass is then washed on the filter with distilled water and then dried in a vacuum or in the incubator at 38° C.

It is then transferred to a flask, and for each centigram 1 c.c. of acetone is added, the mixture being allowed to stand for forty-eight hours with occasional shaking. The acetone is decanted, the bacilli rapidly dried and emulsified in absolute methyl alcohol using a volume equal to the amount of acetone used. This mixture is placed in the incubator at 38° C. for ten days, being shaken three to four times daily. It is then filtered, the filtrate constituting the antigen.

For complement-fixation tests the antigen is diluted with normal saline 1:20.

Before dilution, to dissolve the precipitate which forms on standing, the flask is placed in water at 45 to 50° C. for two or three minutes. The dilution is then made in a clean, dry flask, the saline being added at first drop by drop and later more rapidly.

The resultant emulsion should be opalescent and decidedly opaque.

The reactions with this antigen are said to have a high degree of specificity. Cross fixation with syphilitic serum, however, is said to occur.

The extract in concentrated form was also used for the treatment of experimental tuberculosis in rabbits and guinea pigs and for the production of complement fixing in rabbits.

For this purpose the extract is diluted with an equal volume of distilled water, added at first drop by drop, then more rapidly with constant agitation. The milky emulsion thus obtained is placed on a water-bath at 48-50° C. until all the alcohol is evaporated. One centimeter of the remaining emulsion is equal to 1 centigram of dried bacteria or 5 centigrams of fresh, washed bacteria. For the production of complement-fixing bodies 2 to 3 c.c. are injected into rabbits intravenously at intervals of once or twice a week.

For treatment 1 c.c. amounts are injected intraperitoneally into guinea pigs and subcutaneously into rabbits once or twice a week.

Encouraging results are claimed.

Cunningham, R. S., Sabin, F. R., Sugiyama, S., and Kindwall, J. A.: The Rôle of the Monocyte in Tuberculosis. Bull. Johns Hopkins Hosp., October, 1925, xxxvii, No. 4, 231.

This paper, which is in the nature of a preliminary though extensive report, presents the results of a prolonged study of experimental tuberculosis in the rabbit culminating in the concept that tuberculosis is a disease affecting primarily one single strain of cells, namely, the monocytes.

Evidence is presented to show that in this disease there is a monocytosis (reticular cells, monocytes, and their derivatives, epithelioid and giant cells); that the tubercle bacillus becomes a parasite in the monocytes, and that the predominence of these cells in the lesions is correlated with an increase of monocytes in the circulating blood.

A new avenue of experimental attack is thus uncovered and a new concept of immunity in this disease advanced.

The studies, cytologic in character, were made by a "supravital technic."

Slides and cover-glasses are thoroughly cleaned by immersion for three to four days in concentrated sulphuric acid to which is added a crystal of potassium bichromate; washed in running hot water, then in distilled water, and kept in 80 per cent alcohol. Before use, the alcohol is removed with cheesecloth and the slide thoroughly flamed.

Any trace of grease is fatal to the method.

Saturated alcoholic solutions of vital neutral red and Janus green are diluted for use as follows:

Neutral red: 20 to 30 drops to 10 c.c. of absolute alcohol. The color should be rose-red.

Janus green: 3 drops to 1 c.c. of absolute alcohol is suitable for studies of the blood; for tissue cells, the dye concentration is increased to not more than 6 drops.

The cooled slide is flooded with the mixed dyes thus prepared, the stain being immediately poured back into the bottle. It may be used over and over until contaminated by dust or grease. It must not touch the fingers when poured on the slide or be allowed to remain long enough for the alcohol to evaporate. The slides are dried in an upright position.

Blood is collected on a cover-glass which is inverted on the prepared slide. The preparation is then rimmed with vaseline of high melting point and examined on a warm stage at 38° C.

Lung, liver, and kidneys are gently scraped and mounted as a blood film. The film must be thin. Lymph glands, spleen, and bone marrow preparations are secured with capillary pipettes. Cells from subcutaneous tissues are best secured by producing an artificial edema through the injection of neutral red 1: 10,000 in Ringer's solution and mounting small bits of the resulting gelatinous tissue.

By the study of such preparations the authors believe that they have demonstrated the rôle of the tubercle bacillus in transforming monocytes into epithelioid and giant cells, the pictures seen by them being minutely described and well illustrated.

From their studies the authors conclude that progress in studies of tuberculosis will depend upon attempts to ascertain the mechanism underlying the overproduction of monocytes in tuberculosis and their transformation into epithelioid cells; that the monocyte is the cell primarily affected and which serves as a host to the invading organisms; and that the discovery of the substances affecting them will be the first step in the evolution of a specific immunity.

"With a substance by which the overproduction of monocytes might be checked, then the problem of immunity in tuberculosis might be more analogous to the problem in those diseases which have already been controlled."

Castellani, A.: Biochemical Characteristics of Certain Bacteria When Living in Association or Artificially Mixed and When Living Alone. Brit. Med. Jour., Oct. 24, 1925, No. 3382, 734.

This observation reports some interesting and not readily explainable effects of symbiosis or artificial mixed growths of bacteria upon the biochemical reactions of the organisms.

The B. typhosus, for example, produces acid but no gas when grown in maltose broth. Morgan's bacillus produces neither acid nor gas on the same medium. When B. typhosus and Morgan's bacillus are grown together on maltose broth the expectation would be that only acid would be produced, whereas, as a matter of fact, both acid and gas production occur. The same reaction occurs with mixtures of B. typhosus and B. proteus and with other organisms in mixed cultures as well. It is thus evident that a mixture of two species differing in their biochemical characteristics, when grown together, may produce biochemical reactions common to neither organism. Castellani comments upon the obscurity surrounding this reaction and the necessity for its further study.

Baer, J. L., and Reis, R. A.: The Sedimentation Test in Gynecology. Amer. Jour. Obst. and Gynec., September, 1925, x, No. 3, 397.

The authors report the results of 192 observations on 100 cases. They recommend on the score of simplicity and accuracy, the Linzenmeier-Friedlander technic which in their hands was performed as follows:

Hard glass tubes containing more than 1 c.c. are prepared. The tubes are 6.5 cm. in length and 5 mm. in diameter. Marks are made at the 1 c.c. level and 6, 12, 18, and 24 mm. below this point.

Into a dry Luer syringe is placed 0.2 c.c. of freshly prepared 5 per cent sodium citrate solution and 0.8 c.c. of blood is drawn. The syringe is shaken to mix the blood and citrate and the mixture placed in the tube. The time is taken when the blood is tubed. The tubes are allowed to stand at room temperature and readings are made and the time noted when the line of demarcation has reached the 18 mm. level.

They emphasize the necessity for a uniform technic in order that the results of different observers shall be made comparable. They report their results in the graph below:

Min.	
205	
195	
180	Normal Women
165	Sactosalpinx
150	Uncomplicated Myomata
135	Salpingitis isthmica Nodosa
120	Ovarian Cyst
105	Salpingitis Chronica
90	
75	
60	
45	Salpingitis Subacuta
30	Salpingitis Acuta
15	
5	
0	

Cummer, L., and Lyne, F. R.: The Wassermann Reaction—A System Using an Excess of Amboceptor. Amer. Jour. Syph., Oct., 1925, ix, No. 4, 765.

The authors report their experience with a Wassermann technic devised by Ecker in which the influence of natural antisheep amboceptor in the tested serum is rendered innocuous through the use of an excess of amboceptor in the hemolytic system.

The basic principles of the method involve: (1) The use of an excess quantity of hemolytic amboceptor. (2) The accurate titration of complement in the presence of excess amboceptor. (3) The employment as antigen of a normal heart extract prepared according to the method of Ecker.

Method: Glycerinated antisheep amboceptor is titrated in a range of dilutions of 1:1000 to 1:10,000 or higher; 0.5 c.c. of these dilutions is placed in a series of tubes and 0.25 c.c. of 5 per cent sheep cell suspension, 0.25 c.c. of 1:10 complement added and finally 0.5 c.c. normal saline to make a final volume of 1.5 c.c.

The complement is the pooled serum of several pigs. The tested serum is inactivated at 56° C. for twenty-five minutes.

The antigen is prepared by extracting fresh powdered heart muscle with boiling acetone under a reflux condenser for four hours, cooling in the ice box, filtering, and extracting for the same period in the same apparatus with absolute methyl alcohol, acetone-free, and evaporating to a volume three times the weight of the dried powder.

The cell suspension is sensitized as follows: The amboceptor is diluted so that 1000

units are contained in 50 c.c. of normal saline and this amount added slowly with rotation to an equal amount of 5 per cent cell suspension. After thorough mixing the flask containing the mixture is placed in a 38° C. water-bath for thirty minutes. After this period the flask may be kept at room temperature or in the ice box. In each tube of the complement titration and in the tests, 0.5 c.c. of this sensitized cell suspension containing 5 units of amboceptor is used.

For the complement titer the following amounts of 1:30 complement are placed in a series of 7 tubes: 0.09, 0.12, 0.18, 0.21, 0.24, 0.30, and 0.33 c.c. In each tube place 0.5 c.c. of sensitized cells except the last tube in which 0.25 c.c. of nonsensitized cells is placed, this being the control tube.

Make the total volume 1.5 c.c. with saline and incubate 30 minutes at 38° C. The tube containing the least amount of complement showing absolutely complete hemolysis is the unit. In the reactions 1:10 complement is used and 2 units added to each tube.

The reactions are thus set up: Three tubes, the third being the serum control, are used for each serum. Serum is added as follows: 0.1 c.c., 0.05 c.c., and 0.1 c.c. To the first tube is added 0.1 c.c. of antigen dilution. After fifteen minutes at room temperature, 2 units of 1:10 complement are added to all tubes. Shake and incubate in the ice box for sixteen to eighteen hours (overnight). Then add saline to make a volume of 1 c.c. and 0.5 c.c. of sensitized cell suspension added. Mix and incubate at 38° C. in the water-bath for thirty minutes. Readings are made after a short period to permit partial settling.

For spinal fluids three tubes with corresponding controls are used containing 0.1 c.c., 0.4 c.c., and 0.7 c.c.

The method is stated to be delicate and not to give nonspecific reactions and but few anticomplementary reactions.

Muller, L.: An Apparatus for Anaerobic Cultures. Compt. rend. Soc. de biol. Paris, July, 11, 1925, xciii, 436.

The apparatus is based upon the absorption of oxygen by a heated catalyst. An electric current is not required. The method is designed especially for tube cultures.

A large test tube, 30 cm. × 25 mm. is fitted with a rubber stopper having a central and a lateral perforation.

Through the lateral opening is passed a copper or brass tube 3 to 4 mm. in diameter; through the central opening is passed a copper rod having a diameter of 7 to 8 mm. This rod is about 18 cm. in length and projects into the tube about 6 cm. The external portion is curved at a fairly acute angle. The internal portion is surrounded throughout its length by a fine copper or brass netting which holds a small amount of palladium-coated asbestos. To avoid danger of explosion, this first layer of wire netting is covered by a second of the same material held apart by loosely wound copper wire except at the ends of the cylinder thus formed, where it is tightly fastened by wire ligatures.

The tube should be of such a size that when the apparatus is adjusted there should still be a space about 22 cm. long to receive the culture tube.

In the bottom of the tube is placed a small tube about 2 cm. in diameter half filled with dextrose gelatin containing methylene-blue in the proportion of 1:2000. This serves as an indicator of the degree of anaerobiosis. On top of this is placed a small piece of crumpled filter paper. The indicator tube is placed in the tube first and then the culture tube.

When the culture has been inserted the rubber stopper, moistened with glycerin, is fitted in place, a three-way stopcock is attached to the copper tube by a small piece of rubber tubing and a partial vacuum produced by an air pump. Hydrogen gas is then passed into the apparatus. To start catalysis the curved end of the copper rod is passed through the Bunsen flame several times or inserted into boiling water. After twenty minutes, during which the formation of vapor shows the fixation of hydrogen, the rubber tubing is closed by a pinchcock or plugged with a piece of glass rod and the apparatus incubated.

The complete absorption of oxygen is shown by the decolorization of the indicator (methylene-blue-gelatin).

Howard, H. J.: The Rôle of the Epithelial Cell in Conjunctival and Corneal Infections. Jour. Ophthalmol., December, 1924, vii, 909.

Howard, writing from Peking, in this paper describes his experience with the methods devised by Lindner for the study of eye infections. The gonococcus, pneumococcus, Koch-Week's bacillus, diphtheria bacillus, and influenza bacillus by means of these studies are seen to be parasites of the epithelial cells, proliferating on the surface of the cells and drawing nutriment from them. As they penetrate the deeper layers, they provoke proliferation of the cells and other evidences of reaction. The older epithelial cells covered with a turf of bacteria are cast off; and the younger cells become phagocytes which digest the bacteria.

The methods follow:

The specimen is procured, after anesthetizing the eye with an instillation of 5 per cent cocaine followed by an instillation of 10 per cent cocaine, by gently scraping the palpebral conjunctiva with a platinum spatula.

Dry Fixation: Scrapings are transferred to cover-glasses, allowed to dry in the air, and fixed by flooding with absolute alcohol. The alcohol is allowed to dry spontaneously. The cover-glass is then placed film side down in the staining solution, either Giemsa's stain or Lindner's contrast stain. Giemsa's stain is used in the proportion of 5 to 10 drops to 10 c.c. of distilled water.

Lindner's contrast stain: 10 c.c. distilled water, 5 to 10 drops of Giemsa's stain, 1 drop concentrated alcoholic solution of methylene-blue, and 1 drop of 1 per cent acetic acid solution. Wipe away the metallic scum and mix. Stain for one-half to one hour. Dry and mount in cedar oil—balsam must be avoided.

Wet Fixation: The cover-glass is immediately placed in the solutions indicated. A small tube or jar with rounded bottom so that the film does not come into contact with the container may be used. (1) Ten c.c. concentrated sublimate solution, 20 c.c. 95 per cent alcohol. Thirty minutes. (2) Thirty per cent alcohol two hours. (3) Fifty per cent alcohol two hours. (4) Seventy per cent alcohol, twelve to twenty-four hours. (5) Eighty per cent alcohol to which a few drops of Lugol's solution have been added, twelve to twenty-four hours. The iodine is to remove the mercury. (6) Ninety-five per cent alcohol, twelve to twenty-four hours. (7) Giemsa or Lindner stain. (8) Wash quickly with absolute alcohol. (9) Wash quickly with xylol. (10) Shake off excess of xylol, mount in cedar oil and examine.

Detailed directions are also given for the preparation of sections and the pictures seen are minutely described and well illustrated.

Austin, J. H., and Cullen, G. E.: Hydrogen Ion Concentration of the Blood in Health and Disease. Medicine, August, 1925, iv, 275.

This paper presents a "convenient outline for those whose interests are clinical rather than physiological."

From their own work and the literature the authors summarize the present knowledge concerning the P_H of the blood in health and disease.

Under normal conditions the reaction of the blood is stabilized through the excretion of nonvolatile acids and bases through the kidney, change in the base binding properties of the hemoglobin with oxygenation and reduction, and the excretion of carbon dioxide through the lungs, the last two processes being the more rapid and important.

By the term "P_H of blood" is meant the P_H of the blood serum. Normally this is from 7.3 to 7.4. Colorimetric determination is the method of choice for clinical investigation in the authors' opinion.

Extracellular fluids (edema, nonpurulent joint effusions, etc.) have the same P_H as the serum. Purulent fluids are more acid than serum, ranging from 6.4 to 6.8.

In renal disease marked variations are encountered as might be expected, especially in association with uremia with which varying degrees of acidosis are associated.

In cardiac disease uncomplicated by renal impairment no alteration of moment in the P_H was found. Hyperpnea cannot be explained as a manifestation of acidosis in these cases.

Insulin stupor tends to be associated with an alkalosis and a high P_H. The acidosis of diabetes is, of course, associated with marked changes in P_H. Cases are reported in the literature evidencing recovery from reduction of the P_H to as low as 6.8 to 7.0.

In rheumatic fever there is no evidence of disturbance of the acid-base equilibrium, the plasma and joint fluid P_H being normal.

Dehydration in infants is associated with acidosis.

Fasting for fifty hours produces a depression of 0.02 in the P_H; after seventy-two hours a depression of 0.1 was encountered.

Anoxemia is associated with acidosis especially when due to capillary poisons. Marked fall in P_H occurs in anaphylaxis. A fall occurs in shock when the blood pressure is lowered by shock or hemorrhage; the importance of acidosis as a cause of shock remains to be shown. Changes in the blood P_H (fall) are seen in tetany, but their exact relationship to the disease is not established.

Hemorrhage first causes a drop in the P_H followed by a rise above normal.

In gastric diseases an alkalosis may result from constant loss of HCl and this may also occur after the Sippy treatment for ulcer.

In neoplasms (carcinoma), no significant changes in P_H occur.

Radiation seems to cause a temporary slight (0.02 to 0.09) increase in blood P_H.

In surgical anesthesia there is a combination of acidosis and depressed ventilation. There is, therefore, a constant drop in P_H associated with all forms of surgical anesthesia but satisfactory evidence if its clinical importance is lacking.

In lobar pneumonia no significant changes in the P_H were noted.

In various infections associated with severe febrile reactions a marked drop has been noted in the P_H of the serum.

In pregnancy a slight increase in the P_H has been recorded, probably as a secondary effect of hyperventilation rather than as due to a true alkalosis.

Douglas, S. R., and Meanwell, L. J.: A New Method for the Concentration of Bacilli in Tuberculous Milk. Brit. Exper. Path., Oct., 1925, vi, No. 5, 203.

By the usual methods of examining milk for the presence of tubercle bacilli many of the organisms are carried up with the fat globules and imbedded in the cream layer, while those which remain in the sediment are often in clumps and may thus be missed.

Douglas and Meanwell suggest a method in which these sources of error may largely be avoided.

Ten centimeters of milk to be examined are placed in a centrifuge tube fitted with a rubber-capped screw top. Five-tenths centimeter of liquor trypsin are added and the tubes incubated for three hours at 56° C. or six hours at 38° C. After cooling the tubes are opened, 5 c.c. of ether added, the cap screwed firmly in place, and the tubes shaken thoroughly at least 200 times and subsequently centrifuged for twenty minutes at 4000 r.p.m. After centrifuging three layers will be seen: ether-dissolved fat at the surface, a clear fluid below, and, between the two, a gelatinous disc which contains all the acid-fast bacteria.

A loopful of this gelatinous material is transferred to a slide, a drop of water added and a smear made. After drying at room temperature, the slides are placed into equal parts of alcohol and ether at room temperature for two hours and then stained by the usual Ziehl-Neelsen method. The method has successfully demonstrated tubercle bacilli when present in very small numbers and when the usual methods of examination had failed.

A modification of this method was devised for application to watery fluids such as urine, cerebrospinal fluid, pleural fluids, and the pus of cold abscesses which proved equally satisfactory.

Sputum or pus should first be treated with 20 per cent antiformin for one hour at 38° C. The other specimens are at once subjected to the procedures outlined below.

To 10 c.c. of the fluid to be examined, add 1 to 2 c.c. of oil (olive, cottonseed, or even liquid paraffin). Shake the mixture in order to emulsify the oil as thoroughly as possible. For specimens which have been treated with antiformin, liquid paraffin is more suitable than a vegetable oil.

The emulsion is allowed to stand for ten or fifteen minutes and 5 c.c. of ether is added. After closing the tube with the screw cap, again agitate thoroughly and centrifuge. After centrifuging three layers will be seen: an upper layer consisting of fats dissolved in ether, and a lower layer of watery fluid and debris, the middle layer containing the bacilli.

This intermediate layer varies in thickness, depending upon the character of the fluid in which the organisms were originally suspended. In the case of watery fluids, such as spinal fluid, it may be so thin as to be nearly invisible.

Smears are made and treated as above described for milk.

Where large volumes of fluid may contain only a few bacilli, such as in urine, oil is added to make about 5 per cent of the total volume and the mixture shaken until thoroughly emulsified.

The emulsion is then placed in a separating funnel and allowed to stand until the oil globules have risen to the surface. It is then drawn off and the oily layer run into centrifuge tubes and ether added. After again shaking, the tubes are centrifuged. In this case the oil-ether zone will be found much larger than the watery zone. Films are prepared and stained as above described.

The methods described have been very successful in the hands of the authors.

Osborne, E. D.: Microchemical Studies of Arsenic in Arsenical Pigmentation and Keratoses. Arch. Dermat. and Syph., December, 1925, xii, No. 6, 773.

This paper presents a method for the microchemical study of arsenic in relation to tissue cells and is a variation of that described by Brunauer. It may well be added to the armamentarium of the medicolegal worker.

The technic is as follows: (1) Fixation in 10 per cent formalin (4 per cent formaldehyde), twenty-four to forty-eight hours. (2)Wash in running tap water at lease six hours. (3) Cut tissue in small pieces not more than 2 mm. thick. (4) Place the pieces in a one ounce, ground glass stoppered bottle and nearly fill with fresh, neutral hydrogen sulphide. The stopper is greased with a small amount of petrolatum and tightly fastened in place with tape or cord. (5) Incubate at 70-80° C. for four days, or for six days at 56° C. The latter period better preserves the staining qualities of the tissue. (6) Wash the tissue, which will appear dirty gray to black, in running water six to twelve hours. (7) Dehydrate through 50, 70, 80, 95 per cent and finally absolute alcohol, each containing about 10 per cent of ether. (8) Imbed in celloidin and cut sections not over 5 microns thick. (9) Place the cut sections in 10 per cent HCl (diluted with alcohol), for twenty minutes. This procedure is essential in removing all sulphides except arsenic trisulphide, but it impairs the staining quality of the tissue. (10) Wash in 70 per cent alcohol for twenty minutes and stain in hematoxylin and eosin. (11) Differentiate in 95 per cent alcohol. (12) Clear with oil of cloves, removing the excess with xylol. (13) Mount in balsam. Arsenic trisulphide appears in tissue as nearly round or oval but somewhat irregular greenish-yellow crystals. When in focus the crystals appear solid with a bright halo; too near the objective, they have a heavy rim, a clear center, and a slight halo, beyond the focus they have a clear center, a faint rim and no halo.

Walzer, M., and Kramer, S. D.: Studies in Specific Hypersensitiveness. XVIII: An Indirect Method for Testing for Conditions of Atopic Hypersensitiveness (Preliminary Report). Jour. Immunol., September, 1925, x, No. 5, 835.

Scratch or intradermal tests for sensitization are sometimes confronted with practical difficulties as when, in infants, the cooperation of the patient is lacking or dermographia or other skin lesions interfere with the readings.

The method described is suggested as an alternative method in these types of cases. It is based upon the observation of Prausnitz and Klausner in 1921 that local sensitivity may be passively transferred by injecting into the skin of a normal individual the serum of a sensitized individual and later making skin tests on the normal person thus passively locally sensitized.

Method: When possible, blood is obtained from the presumably sensitized individual by venipuncture. About 4 c.c. of blood is discharged into a centrifuge tube containing sufficient 20 per cent sodium citrate to make a 1 per cent citrated mixture. After centrifugation the serum is pipetted off. Puncture of the heel or toe may be employed, aspirating the blood into a Wright capsule, and the serum obtained by centrifugation.

Where the passive agent is the parent, sterilization of the serum was considered unnecessary; where other individuals were employed, the serum was filtered through a Berkefeld filter.

If only a small amount of fluid was available the serum was diluted with an equal part of normal saline. It is important that the passive agent be free from a history of sensitiveness.

Local sensitization is accomplished by the intradermal injection of $\frac{1}{15}$ c.c. of the serum. About 30 sites 4 cm. apart were used, the anterior and external aspects of the arm and the flexor surface of the forearm being considered the most suitable. The wheals resulting from the intradermal injections are outlined with ink or indelible pencil. While sensitization is complete shortly after the introduction of the serum, the tests are better postponed to the following day.

The actual tests are made by the intradermic injection of about 0.01 c.c. of the antigen solution. Control tests are made at the same time with the same amount on the adjacent normal skin and the substances tested marked on a diagram in order to avoid confusion.

Reactions may be read in five to fifteen minutes. Positive reactions are the same as with the direct method. Erythema around a test in a sensitized site and absent in the control is significant and the test should be repeated on another site. Each site may be tested three to five times provided the same atopen is not used on the same site. Scratch method tests may be used but are not as clear cut as intradermic tests. A general reaction is to be avoided if possible. When it occurs all sensitized sites, tested or untested, may exhibit a marked localized urticaria though the subject is free from symptoms elsewhere. Though sensitivity persists for a month, tests are better expedited, four sittings on alternate days usually sufficing for the testing of a large number of substances.

The method is feasible but now requires study to determine the extent of its practicability.

Mulsow, J.: Culture Mediums for the Gonococcus. Jour. Infect. Dis., April, 1925, xxxvi, 419.

One pound of lean beef and 500 c.c. of water are mixed and allowed to stand forty-eight hours at 38° C. To the expressed juice add peptone 20 gm., potassium nitrate 2 gm., and an equal volume of 0.2 per cent agar melted and cooled to 60° C. Adjust the reaction to plus 0.9 to phenolphthalein. Autoclave fifteen minutes at 15 pounds, determine and adjust reaction if necessary. Best results are obtained when no adjustment of the reaction is required. A P_H of between 6.6 and 7.0 is suitable, 6.8 being preferred.

In this medium the gonococcus will grow as well if not better than contaminating organisms, the growth being confined to the surface.

If smears from the primary culture in this medium show organisms resembling the gonococcus, transplants are made to the differential plating medium described below.

One pound of lean beef and 500 c.c. of distilled water are allowed to stand in the ice chest overnight. To the expressed juice add 10 gm. peptone and 500 c.c. of melted 3 per cent agar cooled to 60° C. Adjust the reaction to plus 0.9 to phenolphthalein. Before the agar has cooled sufficiently to harden, autoclave twenty-five minutes at 15 pounds. Filter through moistened cotton and Canton flannel, place in 100 c.c. flasks and sterilize in the autoclave before the agar has solidified.

When ready to use add to the 100 c.c. flask 0.5 gm. of levulose or maltose and 1 c.c. of 0.4 per cent solution of cresol purple. Melt the agar, cool to 60° C. and add to 50 c.c. of ascitic fluid and pour plates.

Gonococcus colonies are easily distinguished from acid-forming organisms on this medium.

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

*Disease of the Male Organs of Generation**

A CONCISE exposition of diseases of the male generative organs, both functional and organic. There appears an excellent discussion of sterility in the male.

Common Infections of the Female Urethra and Cervix†

THE major portion deals with the venereal infections, but other bacterial diseases of the localities under consideration receive due attention. The authors' discussion of methods of study should be of value to all who presume to undertake pelvic examination. The chapter on prophylaxis is complete and deals with both male and female prophylactic measures. There is a chapter on bacteriology by G. T. Western and one on ophthalmia neonatorum by M. S. Mayou.

The Treatment of Fractures in General Practice††

FOR some time to come the general practitioner will still be called upon to treat fractures. Unfortunately for him, with the development of more satisfactory methods and with the application of the x-ray to this work, the public has come to expect and demand much greater skill and is more prone to hold the physician accountable for unsatisfactory results. It was with a realization of these two facts that the author offered this book to the practitioner. The first portion deals with general principles of diagnosis and treatment and discussion of complications, etc., while the remainder presents in detail the practical treatment of specific fractures. It is well illustrated and clearly and concisely written. Methods have not been simplified, as indeed they cannot be. The reviewer gains the impression that while the book is written for the general physician, it were far better were the treatment of fractures delegated more and more to the trained specialists. It is evidently not the intention of the author to create this impression, but

*Disease of the Male Organs of Generation. By Kenneth M. Walker, F.R.C.S., M.A., M.B., B.C. Cloth. Pp. 234. Price $4.00, Oxford University Press, Henry Frowde & Hodder & Stoughton, London. First printed 1923.

†Common Infections of the Female Urethra and Cervix. By Frank Kidd, M. A., M.Ch., (Cantab.), F.R.C.S. (England) and A. Malcolm Simpson, B.A., M.B., D.P.H. (Cantab.) with additional chapters by George T. Western, M.D., and M. S. Mayou, F.R.C.S. Cloth. Pp. 191. Price $2.50. Oxford University Press. First printed 1924.

††The Treatment of Fractures in General Practice. By C. Max Page, D.S.O., M.S. (Lond.), F.R.C.S. Cloth. Pp. 239. Price $4.00. Oxford University Press, Henry Frowde & Hodder & Stoughton, London. First edition 1923.

we feel it an impression well worth acquiring. Nevertheless, the problem still exists for the man in general practice and he will welcome this handy reference volume.

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*Gynecology with Obstetrics**

A COMPREHENSIVE textbook on obstetrics and general gynecology. Profusely and well illustrated. Little attention is given to the technic of operation—this with the exception of obstetrical conditions. In a prologue, the author gives an interesting historical sketch of obstetrics and gynecology from earliest times. This is followed by anatomic, embryologic and physiologic considerations. The work is primarily a textbook.

Modern Aspects of Syphilis†

MR. HAWKINS presents, in unusually readable form, a concise recapitulation of the problem of syphilis, both diagnostic and therapeutic, as it is interpreted at The Finger Clinic, in Vienna. The viewpoint is exclusively continental and should therefore be of comparative interest to Americans. The author emphasizes the importance of diagnostic lumbar puncture in every case of syphilis. He points out that in the salvarsan treatment of primary syphilis with negative blood Wassermann, a Wassermann must be performed after every injection. If but one time it becomes positive the case then falls into the category of secondary syphilis and requires more prolonged and intensive treatment. Thus, Wassermanns at the beginning and at the ending of the treatment of primary syphilis might be negative while one during the treatment is positive. Such a case would be erroneously diagnosed primary syphilis.

The value of mirion in preventing central nervous manifestations is discussed. The employment of nonspecific measures such as the injection of milk and inoculation with malaria plasmodia, particularly when used in conjunction with specific treatment, is discussed in detail. Intrathecal treatment is evidently not used at the Finger Clinic.

This volume should be highly recommended even to those who treat only an occasional case of syphilis, since it emphasizes the dangers of insufficient early treatment—quite a universal failing among the general practitioners—and presents an excellent routine for comprehensive treatment.

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*Gynecology with Obstetrics. By John S. Fairbairn, M.A., B.M., B.Ch., (Oxon), F.R.C.P. (Eng.). Cloth. Pp. 769. Price $8.00. Oxford University Press. First printed 1924.

†Modern Aspects of Syphilis. By M. J. Morgan, B.A., M.B., B.Ch., B.A.O., N.U.I. Cloth. P. 136. Price $1.75. Oxford University Press, Henry Frowde & Hodder & Stoughton, London. First published 1923.

Books Received

DIE HEILUNG DER TUBERKULOSE und ihrer muschinfektionen (Skrofulose, Rheumatismus, Basedow-krankheit, U. A.). Durch Kutanimpfung von Sanitatsrat Dr. Med. Wilhelm Ponndorf Vorstand der Thuringischen Impfanstalt in Weimar Zweite, Vermehrte und Verbesserte Auflage mit 1 kurve und 1 farbigen tafel. 1923 Verlag von F. C. W. Vogel in Leipzig.

KURZES LEHRBUCH der CHEMIE in Natur und Wirtschaft. Von Prof. Carl Oppenheimer, Dr. phil. et. med. Berlin nebst einer Einfuhrung in die Allgemeine Chemie von Prof. Johann Matula in Wien. 1923. Georg Thieme Verlag Leipzig.

HANDBUCH DER BIOLOGISCHEN ARBEITSMETHODEN. Unter Mitarbeit von 500 bedeutenden Fachmannern von Geh. Med.-Rat Prof. Dr. Emil Abderhalden Direktor des Physiologischen Institutes der Universität Halle a. d. Saale.

Abt. II. Physikalische Methoden, Heft 2. A. Kohler-Jena: Das Mikroskop und seine Anwendung. 1923. Urban & Schwarzenberg.

Abt. III. Angewandte chemische und physikalische Methoden, Teil 7, Heft 5. Pharmokologie, Pharmazie, Toxikologie. W. Storm van Leeuwen-Leiden: Physiologische Wertbestimmung von Giften und Giftkombinationen an Warmblutern und deren Organen. Hermann von Tappeiner-Munchen: Methoden beim Arbeiten mit sensibilisierenden fluoreszierenden Stoffen. Hermann Pfeiffer-Graz: Der Nachweiz photodynamischer Wirkungen flureszierender Stoffe am labenden Warmbluter.

Abt. IV. Angewandte chemische und physikalische Methoden, Teil 10, Heft 4. W. Klein und Marie Steuber-Berlin: Die Methodik des Gaswechselsan grossen Tieren, mit 8 Abbildungen. Paul Hari-Budapest: Elekerische Kompensationscalorimetric, mit 12 Abbildungen. Otto Meyerhof-Berlin-Dahlem: Mikrocalorimetrie mit 14 Abbildungen. J. W. Capstick-Cambridge: Ein Calorimeter für das Arbeiten mit grossen Tieren. Mit 5 Abbildungen. 1925.

Abt. IV. Angewandte chemische und physikalische Methoden, Teil 10, Heft 3. Francis G. Benedict-Boston: Methoden zur Bestimmung des Gaswechsels bei Tieren und Menschen. Mit 74 Abbildungen. 1924.

Abt. V. Methoden zum Studium der Funktionem der einzelnen Organe des tierschen Organismus, Teil 5 A. Heft 2. Methoden der, usket-und Nervenphysiologie. R. Grammel-Stuttgart: Theoretische Grundlagen der Gelebkmechanik. E. Hirt-Munchen: Graphische Methoden zur Darstellung Normaler und Pathologischer Willkurkicher Bewegungsablaufe. Mit 59 Abbildungen im Text. Urban und Schwarzenberg. 1924.

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EDITORIALS

Milk Fever

A DEPANCREATIZED female dog, kept alive by daily injections of insulin and feeding with raw pancreas and sugar, became pregnant and gave birth to five pups. At various intervals during the pregnancy careful observations were made (by W. W. Simpson) of the carbohydrate balance, without finding that any change occurred. As has been pointed out in a previous editorial, this result does not support the view held by some, that the growing fetus can secrete sufficient insulin into the maternal organism to prevent the full development of diabetic symptoms when the pancreas is removed. But a fact of still greater significance observed in this animal, was that marked symptoms of hypoglycemia developed on the day *following* parturition, the cause for which was obviously the removal of glucose from the maternal organism to furnish the lactose of the milk. When this observation was made (January 2, 1925) we failed to realize that it gave the clue to the cause of milk fever in cattle, a fact which has been clearly established through the work of Widmark and Carlens.[1] In the first paper describing their work,

[1]Biochem. Ztschr., 1924, clvi, 453; ibid., clviii, 3, 81.

these authors show that the blood sugar of normal cows while not giving milk is 0.085 per cent, but that it falls to a level very near that at which hypoglycemic symptoms supervene (0.040 per cent) when the animals are being used for milking. A parallelism could also be demonstrated between the height of the blood sugar and the amount of milk which each animal yielded. Subsequently they found that the administration of insulin to normal cows caused the blood sugar to fall to below 0.040 per cent, when symptoms identical with those observed in milk fever supervened; namely, a gradually developing paralysis which ultimately became so severe that the animal collapsed, and although for some time after falling it attempted to raise its head, this finally ceased and the animal lay limp and helpless on its side.

The treatment for this condition obviously demands restoration of the blood sugar and it is significant that this is what occurs when the orthodox veterinary practice of blowing air into the udder is followed. Widmark and Carlens found, in confirmation of earlier work by Paul Bert and Porchet, that this operation causes a prompt rise in blood sugar in lactating animals, most pronounced in those giving the richest yields of milk and of only slight degree in poor milkers. This operation causes mechanical interference with the activity of the secretory cells of the gland, so that they fail to take up glucose from the blood. During the active secretion there is no doubt that amounts of glucose are being carried to the glands which are in excess of those present when no lactation is occurring; the sudden interference with the functioning of the cells then causes glucose to accumulate in the blood until a sufficient concentration is present to act as an inhibitory stimulus to the glycogenolytic mechanism, and so shut down the supply of blood sugar. That such a regulatory mechanism does come into play is indicated by the fact that the hyperglycemia following air injection is only temporary, the normal level of blood sugar being regained in about two hours.

—*J. J. R. M.*

Investigations on the Serodiagnosis of Syphilis

IN 1922, at the Pasteur Institute in Paris, the Health Committee of the League of Nations conducted a six day comparative investigation of the merits of various flocculation reactions in syphilis and the results of the Wassermann test.

Taking part in the investigation were serologists from Austria, Belgium, Denmark, Germany, Great Britain, and Poland.

So divergent were the results obtained that a second investigation and conference was held in Copenhagen, in 1923. Twenty-two workers participated, representing laboratories of London, Warsaw, Copenhagen, Westphalia, Paris, Berlin, Brussels, Heidelberg, and Florence. The United States was represented by two "observers" of the U.S.P.H.S.

The report of this conference is of some interest, first, because it represents a comparatively international effort to approach the specified problems; second, because the flocculation tests were compared, in most instances, by

their originators, and, finally, because of the light it throws on the present status of serologic studies in Europe.

"The main object of these investigations was a comparison between the different flocculation tests and the Bordet-Wassermann test used by the different laboratories."

The flocculation tests studied were: (1) The Sachs-Georgi reaction; (2) the Sigma reaction of Dreyer and Ward; (3) the "Dritte Meinicke reaction"; and (4) Meinicke's "Trubungsreaktion" in which balsam of tolu is substituted for cholesterin in an alcoholic extract of horse heart.

It is of interest that the flocculation tests were performed in most instances either by their originators or by a personal associate thoroughly familiar with the test.

The marked diversity in the technic of the Wassermann test as performed by the different laboratories is in itself a strong argument for some attempt at standardization of technic. A variety of antigen extracts; varying hemolytic systems—antisheep, antihuman, and antigoat; "drop" methods of measuring doses; active and inactivated sera with or without prior absorption of the natural antisheep amboceptor all appear.

There was also a wide divergence in the serum dose and the methods of determining the complement and amboceptor units and, what seems unusual in view of the extensive studies which have been universally conducted relative to the velocity and optimum conditions attending complement fixation in syphilis, the primary incubation was entirely at 37° C. and no mention of fixation in the cold occurs.

During the ten working days of the conference 536 sera and 11 spinal fluids were subjected to examination. Of the sera, 190 were from known syphilis, the remainder being controls from nonluetic conditions, especially tuberculosis, tumors, and pregnancy.

In perusing the report it is difficult to avoid the impression that, subconsciously, there was an effort to "put one's best foot forward"; to show that each of the methods was an excellent method in the hands of its originator or proponent. Indeed, this is, perhaps, one of the valuable features of the report in that alibis are thereby somewhat difficult.

Surveying the problem approached and the imposing titular character of the investigating body, some surprise is felt at the comparatively insular character of the study. There is no mention of the numerous American investigations nor of the modifications of technic arising from them which have done much to add to the sensitivity and reliability of this important and valuable means for the serologic study of syphilis.

Considering the importance of the problem and the relatively enormous area concerned, there seems but one logical or promising method of attacking it.

It should be possible, and is certainly feasible, to select from all the methods proposed those which appear to have outstanding features of excellence. These could then be compared in the endeavor to ascertain if any one, or combination of various features of different methods, could be shown to be superior, and studies could be directed toward determining whether the final method as evolved was satisfactory or possible of still further improvement.

The general conclusions of the committee were, in the main, as follows:

1. The Wassermann test with inactivated sera yielded the greatest number of positive reactions in syphilis and very rarely results possibly nonspecific.

The careful preparation of the antigen extract and the precise determination of the complement dose were held to be of as great importance as the use of multiple antigens. Heart extracts were considered to furnish the best antigens.

2. With active sera a greater number of positive reactions were secured and also a larger number of false positive reactions.

3. "The flocculation tests cannot at present replace the Wassermann test."

As a corollary to this last conclusion, however, the committee generously advises that they all be conducted with the complement-fixation test on all sera.

It cannot be said that the conclusions formulated as a result of these investigations shed any new light upon the problem under consideration, nor does the intention of the committee to retraverse the same ground at a future meeting appear to hold much of promise.

Until the investigations assume a truly international character and embrace the leading serologic methods now in use the standardization of technic will not soon be achieved.

The report contains, as appendices, separate reports in the nature of apologia for the Sigma and other flocculation tests and also of the method of preparing the Bordet-Ruelens antigen extract.

REFERENCE

The Report of the Technical Laboratory Conference held at Copenhagen, November 19, to December 3, 1923: League of Nations Health Organization.

—*R. A. K.*

The Abstract Department

THE Journal has endeavored to maintain at all times a healthy balance between its clinical and its laboratory contributions, and in its editorial work has essayed to correlate the two fields. This we will continue to do.

At the same time the Journal has become, particularly through its department of Laboratory Methods, the standard reference periodical for those whose primary interest is in the field of clinical pathology. In order to provide the clinical pathologist with all of the newer developments of importance within the pages of a single publication, and to enable the clinician to keep abreast of these developments without the expenditure of too much time, the JOURNAL OF LABORATORY AND CLINICAL MEDICINE has instituted a Department of Abstracts devoted to clinical pathology.

In it all articles of value in this domain will be reviewed, but especial attention will be given to descriptions of new methods and apparatus, and improvements in technic. The excerpts will be sufficiently detailed so that it will usually be unnecessary to refer to the original for the carrying out of special procedures.

If the need is felt by the readers of the Journal, the Abstract Department will later be expanded to cover other fields of medicine.

—*W. T. V.*

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The Society maintains a Service Bureau for its members. Any clinical pathologist wishing to make a change should communicate with the Secretary.

Kindly report any change of address to the Secretary.

The next annual Convention of the American Society of Clinical Pathologists will be held in Dallas, Texas, April 15, 16, 17, 1926.